A MODEL FOR STUDY ON HETEROGENEOUS SYNTHESIS OF MOLECULAR FORMS P-450b AND P-450c IN LIVER LOBULE.

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Using monospecific antibodies raised against cytochromes P-450b and P-450c selective induction of these individual monooxygenase isoforms in the microsomal fraction of rat liver has been revealed after consecutive treatment of animals with phenobarbital (PB) and 3-methylcholanthrene (MC) or vice versa. In the liver microsomes of the rats treated with PB under the maximal level of MC-induction the contents of cytochromes P-450b and P-450c were 45 per cent and 54 per cent, correspondingly, of the total content of microsomal CO-binding hemoprotein. The injection of PB followed by MC-treatment resulted in 37 per cent to 63 per cent ratio of cytochrome P-450b to P-450c relative contents. Meanwhile the injection of PB or MC alone caused the relative contents of these isoforms to be 50 per cent and 90 per cent, correspondingly. At the same time the specific catalytic activities of these isozymes towards benzphetamine (P-450b) and 3,4-benzpyrene (P-450c) remained constant in all experimental groups.

By means of immunohistochemical methods a study on liver intralobular location of P-450b and P-450c has been carried out. PB-treatment led to localization of P-450b in the centrilobular region, whereas homogeneous distribution of P-450c in the lobule was observed after MC-treatment. The consecutive treatment with PB followed by MC was accompanied with localization of P-450c only in the cells of periportal region of the lobule. PB-treatment of rats after preliminary MC-induction also resulted in the periportal localization of P-450c, and a small quantities of P-450b was localized in hepatocytes of centrilobular region. Hence, a consecutive treatment of animals with inducers of different cytochrome P-450 molecular forms is accompanied with redistribution of these isozymes in parenchymatous cells of the liver lobule. That is confirmed also by biochemical testing and immunochemical analysis of the microsomal fractions * isolated from hepatocytes of different lobule zones.

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INDIVIDUAL PHASE I- AND PHASE II-DRUG METABOLIZING ENZYMES ARE DIFFERENTLY STABILIZED IN COCULTURES OF LIVER PARENCHYMAL AND NONPARENCHYMAL EPITHELIAL CELLS.

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Liver parenchymal cells rapidly lose their capacity for drug metabolism and other differentiated functions in vitro. Coculturing liver parenchymal cells with nonparenchymal liver epithelial cells is one way to stabilize these cells. Here we report on the effect, that individual drug metabolizing enzymes of the parenchymal cells are differently stabilized in cocultures of the two cell types.

Eight day-old cocultures of liver parenchymal cells with nonparenchymal epithelial cells were assayed for their testosterone hydroxylase-activity, which is a parameter for different cytochrome P450-isoenzymes. Our data show that isoenzymes of the P450-c and P450-p family are stabilized with preference, whereas others are lost totally. Thus, the activity of microsomal epoxide hydrolase (substrate: styrene oxide) is partially maintained and those of cytosolic epoxide hydrolase (substrate: trans-stilbene oxide) and glutathione transferase (substrate: chlorodinitrobenzene) are stabilized well, depending upon the clone of nonparenchymal cells used. These enzyme activities decrease dramatically over a period of 8 days in monocultures of liver parenchymal cells.

Investigating the phase I-metabolism of benzo(a)pyrene in 8 day-old cultures, the metabolite pattern observed in cocultures of parenchymal and nonparenchymal cells was similar to freshly isolated parenchymal cells in suspension (see accompanying presentation by E. Molitor et al.).

These results show that the coculture system of liver parenchymal and nonparenchymal epithelial cells maintain most, but not all of the differentiated functions of liver parenchymal cells. Therefore, we suggest that this system should be used carefully as an in vitro-metabolizing system for compounds of unknown metabolic pathways.

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IMMUNOCHEMICAL ESTIMATION OF THE ROLE OF RAT LIVER CYTOCHROME P-450 FORMS IN PRODUCTION OF MAJOR THEOPHYLLINE METABOLITES

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Recently we showed 1,3-dimethyluric acid (1,3-DMU) production in the reconstituted system with cytochromes P-450IA2 and P-450IIC6 Therefore further immunochemical studies of P-450 participation in production of theophylline (T) metabolites in microsomes of control, PB-, MC- and Aroclor 1254-induced rats is of interest. To this end we used monoclonal antibodies (MAb)made to MC-induced rat liver cytochrome P-450IA1 (MAb 11C10/E11, 15E1/D5) as well as these made to PB-induced cytochrome P-450IIB1 (MAb 12C10/G6) and also polyclonal antibodies to cytochrome P-450IIC6 The monoclonal antibodies had following features: MAb 11C10/E11 recognized in immunoblotting in MC-microsomes P-450IA1 only and inhibited in MC-microsomes O-dealkylation of 7-ethoxyresorufin (ER) by 60% and 7-methoxyresorufin (MR) by 35%; MAb 15E1/D5 recognized in immunoblotting in MC-microsomes P-450IA1 only, inhibited in MC-microsomes O-dealkylation of 7-ER by 80% and did not supress 7-MR-O-dealkylation; MAb 12C10/G6 recognized in immunoblotting in PB-microsomes two P-450 species - P-450IIB1 and P-450IIB2, did not inhibit androstendion 16β-hydroxylation and inhibited 7-penthoxyresorufin-O-dealkylation by 80%.

The antibodies effects on 1,3-DMU production in all species of microsomes are presented in table.

Species of microsomes	Rate without Ab (pmol/min/mg)	Inhibition by antibodies PAb to P-450IIC6	MAb 12C10/G6	MAb 11C10/E11	MAb 15E1/D5
Untreated	26,4 (n=4)	57,4	32,1	27,2	33,0
PB-treated	47,7 (n=4)	63,3	111,3	not studied	160,8
MC-treated	244,2 (n=4)	88,6	13,7	6,4	106,0
Ar-treated	266,4 (n=4)	83,1	27,5	8,6	117,1

3-methylxanthine production was supressed by PAb to P-450IIC6 in control, PB-, MC- and Ar-microsomes by 92, 98, 84 and 60% respectively. MAb 11C10/E11 and 15E1/D5 inhibited 3-MX production in MC- and Ar-microsomes by 33 and 63%, whereas MAb 12C10/G6 by 43 and 92% respectively.

1-methylxanthine production was inhibited by PAb to P-450IIC6 in control, PB-, MC- and Ar-microsomes by 71, 59, 88 and 90% respectively. Quantitative estimation of MAb's influence upon 1-MX production was not possible because of unsufficient resolution of unidentified peak from 1-MX peak.

Thus, immunochemical analysis showed on unexpectedly large contribution of P-450IIC6 to production of all major theophylline metabolites, although in the reconstituted system with this form we obserwed production of 1,3-DMU only. Combination of an immunochemical methods with analysis of drug metabolism in the reconstituted system provides more complete understanding of individual P-450 forms in drug transformation.

EXPRESSION OF DRUG METABOLIZING ENZYMES IN NORMAL AND TUMOUR COLORECTAL SAMPLES FROM HUMAN.

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Colorectal cancer is one of the most frequent cause of death in the Western countries. Until now, little prognostic factors of colorectal cancer are known and studies on chemotherapeutic agents do not demonstrate significant progress in the treatment of this disease.

Epidemiological studies suggest that colorectal cancer is essentially attributed to potential carcinogens and mutagens present in the diet, which, for the most part require metabolic activation to exert their oncogenic effects. Human colonic mucosa possesses enzyme systems able to biotransform xenobiotics and can therefore activate foreign compounds, present in the diet, in ultimate carcinogens in close proximity to their target.

In the present study we investigated phase I (cytochrome P 450 enzymes, DT-diaphorase (DTD)) and phase II enzymes (epoxide hydrolase (EH), glutathione-S-transferases (GSTs), in normal colon from patients without colorectal adenocarcinoma and in peritumoral and tumoral tissues from patients with colorectal adenocarcinoma. Peritumoral tissues were compared either with normal or with tumoral tissues.

No significant changes in levels of cytochrome P 450 IIIA4 (the only one P 450 enzyme detectable in this tissue), EH, GSTs and DTD activity occured between normal and peritumoral tissues. In tumoral tissues, compared to peritumoral tissues, we observed significant decreases of cytochrome P 450 IIIA4 (- 50%, $p<0.01$) and EH (- 60%, $p<0.05$), no change in DTD activity and significant increases in GST pi (+ 40%, $p<0.01$) and in total GST activity (+ 30%, $p<0.05$).

Our results could explain, at least in part, the resistance of colorectal adenocarcinoma to anticancer drugs. Indeed, in tumoral tissues the decrease of P 450 could reduce the efficiency of drugs that require metabolic activation, moreover drugs could be conjugated by increased phase II enzymes before to exert their therapeutic effects. Furthermore, although the colorectal cancer is usually not familial there is an increased susceptibility to colon cancer in familial adenomatous polyposis. So, changes observed in tumoral tissues allow to imagine that variations in drug metabolizing enzymes expression in colorectal adenomatous polyps could be used as pretumoral markers.

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P 1.44

THE INFLUENCE OF THE RADIATION SYNDROME ON CYTOCHROME P-450-DEPENDENT MONOOXYGENATION IN RAT LIVER

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Cytochrome P-450 concentration and the monooxygenation activities of some cytochrome P-450 forms were determined in rat liver in the course of the acute radiation sickness. Ethylmorphine N-demethylation, ethoxycoumarin, ethoxyresorufin, and pentoxyresorufin O-dealkylation were used as indicator reactions. After radiation a distinct decrease in the hepatic biotransformation capacity can be observed, most pronounced at the climax of the radiation syndrome. Phenobarbital treatment (50 mg/kg body mass at 3 consecutive days each) increases the concentration and the monooxygenase activities of some cytochrome P-450 forms. The degree of this induction effect is not altered at the third day after radiation, whereas at day 7 no significant inducibility can be observed.

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POSTERS II (P 2.1 - 2.18): INDUCTION

Source: https://www.industrydocuments.ucsf.edu/docs/sxdl0000

EFFECT OF MIXED-FUNCTION OXIDASE INDUCERS ON HepG2-MEDIATED 1-NITROPYRENE METABOLISM.

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The human liver cell line HepG2, which retains some biosynthetic and metabolic capability of normal human liver parenchimal cells, has been proposed as suitable tool for metabolic and genotoxic studies. In the present work we tested the responsivity of these cells to classic cytochrome P-450 inducers, such as 3-methylcholanthrene (3-MC) and phenobarbital (PB), by evaluating oxidative and reductive metabolism of 1-nitropyrene (1-NP) in control and induced cells.

6- and/or 8-hydroxynitropyrene and 1-aminopyrene (AP) were the most abundant 1-NP metabolites produced by exposure of 1-NP to control cells either as monolayer or in suspension. 3-MC pretreatment increased 35-fold 1-NP oxidative metabolism as compared to control cells. Conversely PB treatment resulted in a 14-fold increase of 1-NP nitroreduction as compared to control cells, whereas it did not affect constitutive 1-NP ring hydroxylase activity.

The role of different cytochrome P-450 form(s) and other microsomal or cytosolic enzymes in 1-NP nitroreduction was evaluated by using homogenate fractions from control and induced HepG2 cells. The contribute of PB-inducible cytochrome P-450 form(s) in 1-NP nitroreduction was established as carbon monoxide and α-napthoflavone inhibited 2.8- and 1.3-fold respectively AP production by PB-induced cell homogenates. Further NADPH showed to be the most effective as electron donor for cellular nitroreductase activity. Cytosolic DT-diaphorase and aldehyde oxidase seemed to be involved, eventhough at low extent, in 1-NP nitroreduction as confirmed by inhibition experiments, whereas the contribute of xanthine oxidase was definitively excluded. Microsomal NADPH-cytochrome P-450 reductase activity resulted to be involved in 1-NP nitroreduction by HepG2 cells, but it was slightly affected by PB-induction of cells.

We can conclude that 3-MC pretreatment of HepG2 cells stimulates 1-NP oxidative metabolism, whereas PB-induction of these cells increases nitroreductase activity, which seems to be associated to a PB-inducible cytochrome P-450 isoenzymatic form(s).

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MORPHINE-INDUCED EFFECTS ON HEPATIC METABOLISM IN THE MALE RAT DEPENDS ON AN INTACT PITUITARY GLAND.

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In analogy with hepatic metabolism of a number of substrates for cytochrome P-450 isozymes, N-demethylation of morphine is sex differentiated and regulated by secretory pattern of growth hormone (GH). Secretion of gonadal as well as of pituitary hormones has been shown to be markedly influenced by morphine administration. Furthermore, administration of opiates to adult rats perturbs the sex-differentiated metabolism of certain drugs and steroids. The aim of the present study was to invetigate whether escalating doses of morphine to adult rats influence N-demethylation of morphine and the role of the pituitary gland in mediating opiate effects on P-450 in rat liver. Increasing daily doses of morphine hydrochloride (20-120 mg/kg, i.p.) to male rats for 2 weeks feminized the sex differentiated hepatic metabolism of 4-androstene-3,17-dione (androstendione) and N-demethylation of morphine in vitro. The combined treatment by human growth hormone (hGH) and morphine led to a pronounced effect on N-demethylation of morphine in male rats. Only minor effects of morphine on the liver metabolism of these two substrates were observed in female rats. The total content of P-450 in liver microsomes decreased significantly following morphine treatment of males and females and in males receiving hGH and morphine. Concomitant administration of naloxon hydrochloride (1-4 mg/kg) and morphine did not alter the feminizing effect of morphine on androstendione metabolism in intact male rats. In hypophysectomized rats,however, morphin or morhine/naloxon tratment did not affect the pattern of androstendione metabolism as compared to hypophysectomized rats. Data obtained in the present study clearly show the necessity of an intact pituitary gland as a mediator of morphine-induced effects on sex differentiated hepatic metabolism in rat.

COMPARATIVE EFFECTS OF THREE FLAVONOIDS AND FOUR KNOWN INDUCERS ON DRUG METABOLIZING ENZYMES IN RAT LIVER.

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The effects of 3 flavonoids - flavone, flavanone and tangeretin - (2000 ppm of the diet), were studied on enzymatic activities in rat liver. Ethoxyresorufin dealkylase (EROD), pentoxyresorufin dealkylase (PROD), erythromycin N-demethylase (ERDM) and laurate hydroxylase (LH) activities were measured, as representative monooxygenase isozymes of cytochromes P-450 IA, P-450 IIB, P-450 IIIA and P-450 IV respectively, which are the most common cytochrome P-450 families found in rat liver. The glutathione transferase (GSHT) and the UDP-glucuronyl transferases (UDPGT1 and UDPGT2) activities were assayed as conjugation reactions. Cytochrome P-450 content and NAD(P)H-cytochrome c reductases activity were also measured.

The 3 flavonoids had no effect on the cytochrome P-450 level but they were found to modify the enzymatic activities. They had a low effect on NAD(P)H-cytochrome c reductases activity but they differentially affected activities of monooxygenase and transferase: flavone increased activities of EROD (3-methylcholanthrene inducible), PROD (phenobarbital inducible) and of conjugation reactions whereas flavanone enhanced activities of ERDM (dexamethasone inducible), GSHT and UDPGT1. Tangeretin increased PROD and, to a lesser extent, EROD, ERDM, LH (ciprofibrate inducible), UDPGT1 and UDPGT2 activities. In contrast, this flavonoid did not modify GSHT activity.

Preliminary identifications of induced cytochromes P-450 by immunoblotting were achieved using antibodies of cytochromes P-450 induced by 3-methylcholanthrene (P-450 IA) or by phenobarbital (P-450 IIB). Results indicated that flavone and tangeretin increased forms of cytochrome P-450 IA and IIB wheras flavanone induced only the cytochrome P-450 IA isozyme. It is concluded that flavonoids tested acted differentially and specifically on drug metabolizing isozymes in rat liver, their inducing characteristics being dependent on their chemical structures.

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REGULATION OF THE INDUCTION OF DRUG-METABOLIZING ENZYMES IN GUNN RAT LIVER : IMPLICATION OF THE INTRACELLULAR HAEM POOL.

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Gunn rat strain is known for its unconjugated chronic hyperbilirubinaemia. We have recently shown (1) that, unlike Wistar, Gunn males did not exhibit changes in the overall cytochrome P-450 content of hepatic microsomes after administration of 3-methylcholanthrene (MC). This apparent lack of induction is due to a balance between the rise in P-450 c and d (IA1 and IA2) and decrease in P-450 2c (IIC11) as shown by immunoquantification. In contrast, the response of male Gunn rats to phenobarbital (PB) administration was similar to that observed in Wistar rats. So we have postulated that haem availability might be the limiting step in the regulation of P-450 isoenzymes synthesis in the Gunn rat.

According to Badawy et al.(2), the haem pool can be evaluated by measuring the tryptophan pyrrolase activity. The results show a substantial reduction in tryptophan pyrrolase activity in control Gunn versus control Wistar rats (-56%). Furthermore MC treatment emphasizes this difference by lowering the haem pool in Gunn rats but has no effect in Wistar rats (tryptophan pyrrolase activity in MC-treated represents 50 % of control Gunn activity). Adversely PB treatment abolishes the differences between both strains increasing activity in PB-treated Gunn rats to that of control Wistar rats (+200%).

Taken together, our results show that PB and MC can exert opposite effects on the haem intracellular pool. These data are in agreement with our previous reports and confirm that the variations of the intracellular haem pool can lead to a stimulation or to a limitation of the synthesis of cytochrome P-450 isoenzymes in the Gunn rat.

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2028903647

EFFECTS OF AGEING, GENDER, AND PHENOBARBITAL TREATMENT ON GLUTATHIONE S-TRANSFERASE ACTIVITY AND SUBUNIT COMPOSITION OF RAT LIVER

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Glutathione S-transferase (GST) consists of a family of multifunctional isoenzymes, catalyzing the conjugation of a wide variety of hydrophilic electrophiles with glutathione. Although some studies concering GST activity of rat liver have been carried out with respect to age, it is not yet clear whether GST undergoes important changes or not in function of ageing of the animals. Only male rats have been involved and no data on the isoenzyme pattern after phenobarbital (PB) treatment are available.
Therefore the GST activity and the subunit pattern of male and female Brown Norway rats (BN/BIRIJ) of different ages (3, 6, 12, 18, 24 and 36 months), before and after PB treatment (45 mg/kg body weight . day for 7 days), have been studied in liver tissue. The GST activity was measured spectrophotometrically using CDNB and DCNB as substrates.
The subunit pattern was determined by purification of the GST isoenzymes by affinity chromatography followed by reversed phase HPLC.
From the results obtained it generally appeared that the most important changes in GST activity and subunit pattern are concerned with gender and PB treatment and not so much with ageing.
As far as the gender of the animals is concerned, it was found that, for all age groups studied, male livers showed significantly higher GST activities than females did and this was the case not only under control conditions but also after PB treatment. It seemed that especially subunits 3 and 4, predominantly present in male rat liver, were involved.
After PB treatment, shifts in the percentage subunit composition occurred in both sexes, making the differences between male and female animals even more evident. The absolute amounts of all subunits were induced. This was particularly obvious for subunits 1 and 3 in both genders but in addition only in male rats subunit 2 was strikingly increased.
When ageing was studied, small but significant changes in GST activities were observed for both substrates. It seemed that the GST activities of male and female livers tended to converge in function of age, although, for all age groups studied, the subunit pattern remained constant under control conditions and after PB treatment.
In conclusion, from this study it appears that glutathione conjugation of liver is very depending on the gender of the animals and on inducing factors such as PB. With ageing, rather small changes are involved, indicating that, as far as GST is concerned, no important changes have to be expected in the phase II metabolic capacity.

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MODULATION OF CIPROFIBRATE INDUCIVE EFFECTS ON THE HEPATIC DRUG METABOLISM ENZYMES AND ON THE PEROXISOME PROLIFERATION IN THE RAT IN VARIOUS THYROIDIC STATUS.

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We studied the influence of thyroid hormone treatment or thyroid hormone suppression on the fibrates-induced peroxisome proliferation via a modulation of microsomal cytochrome P450-IVA1 content.

Fibrates are known to induce microsomal cytochrome P450 IVA1 and to produce hepatic peroxisome proliferation with induction of the CN⁻insensitive Palmitoyl CoA oxydase (PCAO), carnitine acetyl transferase(CAT) and bifonctional enzyme enoyl CoA hydratase (ECH).

The laurate hydroxylase cytochrome P450 IVA1 dependant induction, followed by dicarboxylic acids formation, could induce this proliferation, as suggested by Lake et al.

Male Wistar rats in various thyroidic status were treated with ciprofibrate (5mg/kg body weight/day during 8 days). A significant decrease of lauric acid hydroxylase activity (cytochrome P450-IVA1) in hypothyroidic animals as well as in hyperthyroidic animals, when compared to controls, suggested a modulation of ciprofibrate inducive effects on microsomal and cytosolic enzymes. This decrease of the inducive effect related to fibrates also occured at the level of bilirubin-UDPGT and cEH.

For the peroxisomal enzymes, the moderation of the fibrates inducive effect was more important in hypothyroidic animals (a 39%, 35% and 13% decrease of the inducive effect in regard to respectively PCAO, CAT, and ECH) than in hyperthyroidic rats (a 31%, 28%, and 5% decrease of the inducive effect with regard to respectively PCAO, CAT and ECH) was observed.

In conclusion, the inducive and proliferation effects of ciprofibrate seems to be, in some extend, dependant of the thyroid state of the experimental animals.

20289036 48

EXPRESSION OF CYTOCHROME P-450b,e GENES BY COINDUCTION WITH PHENOBARBITAL AND TCPOBOP.

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1,4-bis-2-(3,5-dichloropyridylary)benzene is known to induce the PB-family of P-450 cytochromes in mice liver, but in rats it does not cause the expression of the PB-family of genes. We have studied the influence of the coinduction of phenobarbital (PB) and TCPOBOP on the expression of P-450b,e genes in the liver of Wistar rats.
Two induction schemes have been used. In the first one male rats were treated with PB at a dose of 20 mg/kg simultaneously with a usual dose of TCPOBOP (3mg/kg). In the second one animals were treated for two days with PB, on the third day were subject to simultaneous injection of PB and TCPOBOP. In both cases rats treated with PB only served as controls. Microsomes were obtained 96 hours after the first treatment.
Metabolism of such substrates as pentoxyresorufine, benzphetamine and androstendione has been used to estomate catalytic activity of cytochrome P-450b. In both schenes increase of oxidation rates has been observed for above substrates although it was not as pronounced as in case of standard PB-induction. Total mRNA content for cytochromes P-450b,e has been estimated by hybridization with cDNA for cytochrome P-450e and with olygonucleotide probes for mRNA of cytochromes P-450b and P-450e. Increase of total mRNA content for P-450b,e correlated with variations in catalytic activity of the microsomal monooxygenase system as well as with the increase of the total cytochrome P-450 level in microsomal system.
Thus the induction of rat liver monooxygenase with TCPOBOP has been observed for the first time. This can suggest that for the expression of cytochrome P-450b,e genes the proteins P-450b,e should be initially present in liver microsomes.

Source: https://www.industrydocuments.ucsf.edu/docs/sxdl0000

THE INFLUENCE OF VARIOUS INDUCING AGENTS ON METABOLISM OF DIAZEPAM BY RAT LIVER MULTIPLE FORMS OF CYTOCHROME P-450

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Various classes of inducers selective induce isozymes representing at least four different cytochrome P-450 subfamilies in rat liver. Human and experimental animals livers contain a group of immunochemically and catalytically related cytochromes P-450 exemplified by P-450p (rat), LM3c (rabbit) and HLp (man), inducible with phenobarbital, "phenobarbital-like compounds", macrolide antibiotics, synthetic glucocorticoids and antiglucocorticoids and antifungal agents. The study was performed to access the effect of phenobarbital (PB), dexamethasone (DEX) and clotrimazol (CL) induction on rat liver microsomal activity towards to diazepam to clarify participation of some P-450 isozymes in biotransformation of this substrate. The in vitro metabolism of diazepam to nordiazepam, 3-hydroxydiazepam, oxazepam and androstenedione to 7α-, 16β-, 16α-, 6β-hydroxyandrostenediones was studied using intact male and female rat liver microsomes and after PB (100 mg/kg), DEX (100 mg/kg) and CL (100 mg/kg) induction during 3 days and were determined by HPLC. We were revealed a large intersexual differences in diazepam biotransformation (and also in androstenedione stereo- and regioselective hydroxylation) as by microsomes of intact rats as of induced animals. These results let us to suppose involvement of sex-specific isoenzymes of cytochrome P-450 in biotransformation of this benzodiazepine tranquilizer. PB-treatment of rats causes a 5-8-fold enchancement in diazepam metabolism rate in liver microsomes of males and females. These data are in a good agreement with a high activity of purified cytochrome P-450b in formation of nordiazepam from diazepam and oxazepam from nordiazepam as a substrate (our experiments in reconstituted system). Microsomal preparations from animals treated with DEX and CL extensively metabolized diazepam (especially, on the way of 3-hydroxydiazepam formation) and androstenedione (demonstrating a high increase of 16β- and 6β-hydroxyandrostenediones production), and CL was more strong inducer of these catalytic activities compared to DEX. Microsomal preparations from intact and MC-treated rats generated only low levels of metabilites of diazepam and androstenedione. In conclusion, our results of experiments in reconstituted system demonstrate that cytochromes P-450a, P-450c and P-450d were not involved in diazepam metabolism, whereas P-450b catalyses its N-demethylation with the following hydroxylation of the primary metabolite; while P-450p accounted for most of the form involved in 3-hydroxydiazepam production and 6 -hydroxylation of androstenedione. Further experiments both in vivo and in vitro have to be carried out on animals and man to understand the possibility of diazepam using as a "test" drug for the estimation of cytochrome P-450 multiple forms functioning and induction.

2028903649

Biological activities of polychlorinated dibenzo-p-dioxins (PCDDs) in rat hepatocytes and rat hepatoma (H4IIE) cells: Comparison between defined mixtures and their constituents.

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PCDDs are widespred environmental pollutants which are present as complex mixtures with widely differing toxicity of their constituents. A remarkable correlation was found between the binding affinity of PCDDs to the Ah receptor, the induction of P450IA1 (measured as 7-ethoxyresorufin O-deethylase = EROD) and various toxic effects of PCDDs in vivo (1, 2). However, the assessment of biological activities of complex PCDD mixtures still represents a great challenge for toxicology.

We determined dose-response curves of EROD induction by defined mixtures containing 49 PCDDs and by their 2378-substituted constituents. EC_{50}-values were calculated by log-probit analysis and compared with the EC_{50}-value of the most potent congener, 2378-Cl_4DD (2378-tetrachlorodibenzo-p-dioxin). They were expressed as 2378-Cl_4DD equivalents (TEs). For 2378-substituted PCDDs the following TEs were obtained:

2378-Cl_4DD:	1.00
12378-Cl_5DD:	0.18
123478-Cl_6DD:	0.18
123789-Cl_6DD:	0.06
123678-Cl_6DD:	0.04
1234678-Cl_7DD:	0.05

Similar TEs were obtained with hepatocytes and H4IIE cells. TEs of two defined PCDD mixtures could be predicted from the sum of TEs of the 6 most potent 2378-substituted constituents which accounted for only 13 or 20% of total PCDDs, i.e., biological effects of the major portion of the non-2378-substituted PCDDs could be neglected, and no antagonistic and overadditive effects were found. However, for Cl_8DD and non-halogenated polycyclic aromatic hydrocarbons much higher EC_{50}-values but lower efficacies (maximal enzyme activities) were found, the latter findings making comparison of TEs difficult.
The results suggest that (a) TE-values determined in H4IIE cells were similar to those found in hepatocytes, and (b) biological effects of PCDD mixtures were mostly due to additive effects of six 2378-substituted congeners.

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THE EFFECT OF ANTIEPILEPSIRINE ON THE CYTOCHROME P-450 RAT LIVER MICROSOME

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Antiepilepsirine (AES), 3,4-methylene dioxycinnamoyl piperidine, is an antiepileptic drug. In this paper, the effect of AES on the cytochrome P-450 in rat liver microsomes has been studied.

Rat liver microsomes was prepared by methods of Ca^{++}aggregation or ultracentrifugation. The plasma concentration of AES was measured by HPLC.

The liver weight, microsome yield and cytochrome P-450 content after a single oral dose of AES (ig) were higher than those in control group and after a single dose of AES intraperitoneally (ip), but these effects were much weaker than PB.

Tab. Effects of AES and Phenobarbital (PB) on the microsome yield and cytochrome P-450 in rat liver (n=6)

Drug	Dose (mg/kg)	Microsome yield (mg/g liver) ip	Microsome yield (mg/g liver) ig	P-450 content (nmol/mg pr) ip	P-450 content (nmol/mg pr) ig
Control		10.6±4.2	9.7±1.3	0.77±0.22	0.90±0.41
AES	100	10.9±4.1	9.8±3.3	0.95±0.45	1.29±0.35
AES	200	11.2±4.0	12.0±1.8*	1.19±0.52	1.53±0.44*
PB	60	14.5±3.8	14.9±1.7**	1.90±0.28	2.70±0.65**

P<0.05, P<0.001

Meanwhile, a decrease of AES plasma concentration induced by AES and PB was observed. It is suggested that AES as well as Phenobarbital and other antiepileptic drugs exhibits a drug enzyme induction and autoinduction in rats liver microsomes.

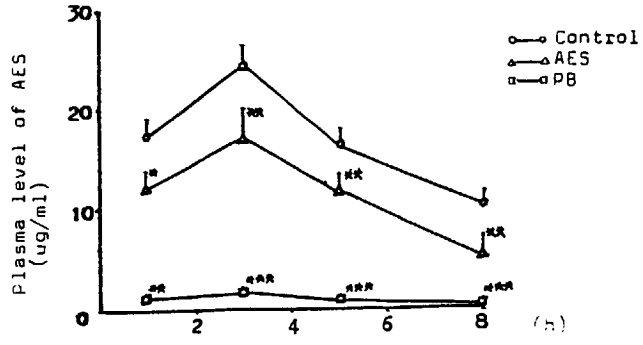

Fig. Effects of AES and PB on the plasma concentration of AES in rats (n=5)

DIRECT LABELLING OF RAT LIVER CYTOCHROME P450IA1 (P-448) BY 3H-AR-L 57

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The tritium labelled cardiotonic agent AR-L 57 was used in binding assays with rat liver microsomes. 3H-AR-L 57 binds reversibly and specifically to liver microsomes with high affinity (K_D = 60 nM). Gassing of microsomes by CO prior to the binding assay reduced the number of binding sites. The bound radioactivity was displaced by structurally related cardiotonics like sulmazole and other imidazopyridines as well as by known substrates or ligands of cytochrome "P-448". 3-methylcholanthrene (MC), 3,4-benzo(a)pyrene (BP), and ß-naphtoflavone (NF). In contrast to this result phenobarbitone did not influence binding. After pretreatment of rats p.o. with sulmazole, BP, NF or MC a large increase of the number of binding sites was found. The administration of phenobarbitone was without significant effect. These microsomes were also monitored for ethoxyresorufinedeethylase (EROD) activity showing parallel results.

Tab.: Increase of the number of binding sites for 3H-AR-L 57 and EROD-activity after pretreatment with enzyme inducers and pKi values of these compounds for inhibition of 3H-AR-L 57 binding.

compound	EROD	sites	pKi
3-MC	86	38	8.0
ß-NF	76	29	8.5
sulmazole	4	5.4	6.7
AR-L 57	1.3	2.0	7.4
8-MOP	5.5	5.7	6.5
isomazole	5.5	5.5	6.1

Furthermore, AR-L 57 is a potent inhibitor of EROD- activity. Finally comparing the binding of the enantiomers of sulmazole with their pharmacokinetics we could demonstrate a 10 times higher affinity of the (-)enantiomer which is reflected by an increased clearance of this enantiomer in vivo.

Conclusion: From the parallel augmentation to binding sites with deethylase activity the identity of these sites with cytochrome P-448 is very likely. Binding assays may be a useful tool to measure enzyme induction. It furthermore may help to elucidate the rate-limiting step of the whole P-450 mediated reaction cyclye.

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INCREASED EXPRESSION OF COUMARIN 7-HYDROXYLASE BY COBALT CHLORIDE, PYRAZOLE AND PHENOBARBITAL IN MOUSE LIVER

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The multigene family of heme-containing cytochrome P450 isozymes catalyzes the metabolism of a multitude of exogenous and endogenous compounds. Heavy metals usually decrease the ability of the P450 system to metabolize xenobiotics by decreasing the amount of P450 and activities associated with it, probably by interfering with heme metabolism. In contrast to this general rule, it has been reported that cobalt selectively increases the metabolism of coumarin and its derivatives in mouse liver.
Several heavy metal salts were tested for their effects on mouse liver P450-dependent monooxygenase reactions. Most metal ions (tin, copper, platinum, gold, lead) had no marked effect or a depressing effect on cytochromes b5 and P450 and associated activities. In contrast, cobalt chloride administration caused a substantial increase in the activities of COH and 7-ethoxycoumarin O-deethylase (ECOD) in DBA/2 and C57BL/6 mouse liver, as also did pyrazole, a prototype COH inducer, and phenobarbital. The activity of COH has been shown to be supported by a single P450 isozyme, designated as P450Coh. Immunoblot analysis with anti-P450Coh antibody showed that increases occur also in the P450Coh protein content in response to treatment with cobalt, pyrazole and phenobarbital. Using the previously isolated cDNA clone encoding for P450(15α) (from Dr. Negishi, NIEHS) it was shown in Northern blot analysis that a 2.1-kb mRNA message is increased by pyrazole, phenobarbital and cobalt. RNA slot-blot analysis showed that hepatic cDNA-hybridizable mRNA is increased by cobalt to roughly the same extent as COH activity and P450Coh content in both mouse strains. The amount of hybridizable mRNA is disproportionally induced in the DBA/2 strain by pyrazole. A portion of the increase may be due to the increase in the mRNA of a closely related P450 form catalyzing testosterone 15α-hydroxylation. These data suggest that cobalt, phenobarbital and pyrazole increase the expression of the P450Coh system, although it is not yet known whether the expression is increased by a transcriptional and post-transcriptional mechanism. This system provides an excellent model to study the regulation of heavy metal-induced changes in P450 expression.

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ANTAGONISTIC EFFECT OF PRISTANE ON CLOFIBRATE INDUCED FATTY ACID HYDROXYLATION

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In a recent work [(1)] it has been demonstrated that the isoprenoid hydrocarbon pristane (2,6,10,14 tetramethylpentadecane) undergoes subterminal hydroxylation or terminal oxidation followed by the classical β-oxidation process. Moreover, Ikeda et al [(2)] observed that peroxisome proliferation in rat liver was induced by dietary administration of 2,2,4,4,6,8,8-heptamethylnonane, a highly branched alkane in a way similar to the one encountered with hypolipidemic drugs, and very likely via its major metabolite detected in liver which is the α-disubstituted carboxylic acid.

In the present experiment we analysed the relative effects of pristane, of clofibrate (model of peroxisome proliferation) and of these two xenobiotics in association, on rat hepatic cytochrome P450 levels, and on two microsomal enzymatic activities : alkane hydroxylation and fatty acid hydroxylation.

If no significant modification of body weight was observed in treated rats, in contrast, the relative liver weights were significantly larger than those of control rats (15%, 23% and 41% increase respectively in pristane clofibrate and pristane + clofibrate groups). The same response was observed for cytochrome P450 contents (19%, 28% and 96% increase respectively).

When considering hydrocarbon hydroxylation, it was observed that clofibrate reduced this enzymatic activity in the same way whether it was distributed alone or associated with pristane. Moreover, this phenomenon was correlated with the observation that at slaughtering, residues of pristane in rat adipose tissue were lower (500 ppm) in pristane group than in pristane + clofibrate group (700 ppm).

Regarding the effect on fatty acid hydroxylation, it has been confirmed that clofibrate was an efficient inducer for laurate hydroxylase and in a lesser manner for palmitate hydroxylase. Activity of this latter enzyme is not modified by pristane, which nevertheless is responsible of an induction of laurate hydroxylase (2.3 fold). When pristane and clofibrate were associated in the rat diet, it appeared that clofibrate effect was completely inhibited since the responses on fatty acid hydroxylation obtained are similar to those measured in pristane group.

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EFFECT OF TRANS-STILBENE OXIDE ON THE REGULATION OF RAT GLUTATHIONE S-TRANSFERASE ISOENZYMES IN VIVO

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Glutathione S-transferase (GST) forms a group of multifunctional proteins all of which catalyze the conjugation between glutathione and a wide range of hydrophobic electrophiles. As is the case for other xenobiotic metabolizing enzymes, this group of isoenzymes can be modulated by internal and external factors. The effect of some inducers on the GST activity, i.e. phenobarbital and methylcholantrene, is well studied in vivo, but very little is known about the influence of trans-stilbene oxide (TSO).
In this study, male Sprague-Dawley rats (180-200 g) were injected (IP) with 600 mg TSO/kg during 5 days. Corn oil (used to dissolveTSO) injected rats were used as controls. GST activity increased 2.9 and 2.0 fold towards the substrates 1-chloro-2,4-dinitrobenzene and 1,2-dichloro-4-nitrobenzene respectively. Purification of affinity chromatography and separation by HPLC of the different GST subunits revealed the appearance of subunit 7, absent in normal non-treated liver and detected in hepatoma's. Furthermore a specific effect of the epoxide on subunits 1, 2 and 3 was observed. Subunit 4 remained unchanged.
Immunocytochemical analysis demonstrated a homogeneous staining of the treated liver for subunit 7 and a change from a heterogeneous to a homogeneous staining for subunits 1 and 3. By northern blot analysis a more important increased steady-state mRNA level was observed for subunits 1/2 than for subunits 3/4 when TSO-treated samples were compared with controls. By the same technique, the presence of subunit 7 mRNA was confirmed.
"Nuclear run-on" experiments proved the induction of subunits 1 and 3, and the appearance of subunit 7 mRNA.

POSTNATAL DEVELOPMENT OF THE MIXED FUNCTION OXIDASE SYSTEM IN JAPANESE QUAIL AND INDUCTION BY PCB.

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In the environment, birds are more and more exposed to a large variety of pollutants (pesticides, industrial pollutants...). Most of these products are metabolized by the hepatic microsomal monooxygenases. Unfortunately, some of these pollutants such as polychlorinated biphenyls (PCB) may accumulate in different tissues and namely in the liver. As these products are known as inducers of the cytochrome P-450 dependent enzymes, they may modulate not only the metabolism of xenobiotics but also that of endogenous compounds like steroid hormones. PCB have namely been shown to affect the laying of Japanese quails.

The present study was initiated to determine the postnatal development of the cytochrome P-450 dependent enzymes in Japanese quail and the induction of this system by PCBs.

Japanese quails received PCB, per os, each 48 hours during 48 days at a dose of 50 mg/Kg. Every two days, birds were killed, their liver removed to prepare microsomes. In addition, eggs from these contaminated quails were incubated and the livers were removed from 9th day old fledgling for analysis. Microsomal protein concentrations, cytochrome P-450 content and aldrin epoxydase activity and PCB content have been determined on these liver samples.

In untreated birds, microsomal protein concentrations remain stable from the 6th up to 48th day of life. No important modification of P-450 concentrations were observed during this period. In adult animals, a concentration of 1 nmole/mg microsomal protein is reached. Aldrin epoxydase activity decreases on the 27th day to stabilize around 15 pmoles/mg protein.

The administration of Aroclor 1254 induces both cytochrome P-450 and aldrin epoxydase. Already after the first day of treatment, P-450 is induced by a factor 5 and aldrin epoxydase by a factor 50. After 18th day of treatment, P-450 concentrations decrease to reach a level still 2 times higher than that measured in the untreated animals.

On the other hand, no induction was observed in young quails originating from intoxicated parents.

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CONTRIBUTION OF mRNA LEVEL DETERMINATION IN INDUCTION PROFILE OF DRUG METABOLIZING ENZYMES.

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New pharmacologically active compounds may modulate the activities of the drug metabolizing enzymes and thereby alter their therapeutics effects. For that reason, it is of great importance to determine an accurate induction/inhibition profile of these molecules as early as possible at the preclinical stage. We have carried out a method of mRNA level quantification concerning mainly cytochromes P-450 and compared the results to the corresponding enzymes activities previously determined, and also to some immunochemical data.
In this study, we applied this technique to RP 52028, an antagonist of peripheral type benzodiazepine binding sites, known to have induction properties in rat and human. Dose-dependent effects of RP 52028 were studied on mRNA levels in male and female Sprague-Dawley rat livers, and compared to those of a reference molecule used in induction studies, phenobarbital.
RP 52028 showed an induction pattern quite similar to phenobarbital, but appears to be less potent, considering the results obtained for the low dose treatment (which is equimolar to the PB dose). PB-inducible isoforms (Cyt P-450 IIB1+2 and IIIA1) were increased in both sexes. However, we found a sex related difference in responsiveness to treatment by both molecules, since female rats exhibited a more important inducibility (induced to control ratio) of these isozymes.
As for constitutive isoforms (Cyt P-450 IIC7 and IIC11), their mRNAs were expressed at a high level in control animals in a sex-specific manner, but barely changed in treated animals.
Some of these results do not correlate with enzymatic and immunochemical data. These discrepancies will be discussed in terms of lack of substrate specificity or regulation mechanism plurality.

RP 52028 was kindly provided by Rhone-Poulenc Santé.

A PROPOSED MECHANISM FOR INDUCTION OF CYTOCHROME P-450IA1 mRNA IN RAT COLON BY INDOLE-3-CARBINOL AND 5,6-BENZOFLAVONE.

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Cruciferous vegetables have a protective effect on the induction of cancer by chemical carcinogens in animals experiments. Indole-3-carbinol (I3C) is suspected to be one of the active components (1), but the mechanism of action remains unclear. It has been suggested that I3C 1) induces the cytochrome P-450 (P-450) enzymes, 2) influences the phase II enzymes and/or 3) acts as a scavenger for the ultimate carcinogen.
We have previously shown that both I3C and 5,6-benzoflavone (5,6BF) induced P-450IA1 mRNA in rat colon and liver and induce P-450IA2 mRNA in liver of rats by a single per oral (p.o.) treatment (2). Furthermore, this treatment showed a biphasic profile of mRNA levels with peaks at 4 h and 16 h in colon. This has also been observed in fetal mouse lung after treatment with 5,6BF (3). In rat liver a single broad induction peak around 12 h was observed (2).
There are two plausible explanations for the bimodal profile: 1) The peaks in the P-450IA1 mRNA accumulation corresponds to the different routes by which the inducer reach the colonic epithelium, by the blood supply (4 h) or via the intestinal tract (16 h). 2) The peak at 4 h is mediated by the compound itself and the peak at 16 h by a metabolite.
To evaluate the underlying mechanism for the biphasic induction profile of P-450IA1 mRNA in colon, we measured the time dependent mRNA level in rat colon and liver after a single intra-rectal (i.r.) administration of 5,6BF and I3C, together with the P-450IA1 protein level in liver.
A single induction peak around 4 h would result after i.r. administration, if different routes caused the biphasic profile found in the p.o. experiment, whereas an induction profile identical to the p.o. administration would appear, if the two peaks were caused by the inducers and their metabolites, respectively.
After a single i.r. administration of 5,6BF, a major peak of P-450IA1 mRNA was observed at 2 h in colon and a minor (and insignificant) peak at 12 h. This show that the bimodal profile found after p.o administration is caused by different routes by which the inducer reach the colon.
Intrarectally administered I3C caused an insignificant increase of P-450IA1 mRNA at 12 h. This lack of inducibility was also found following intraperitoneal administration of I3C, because I3C needs to be converted to an active form in the acid environment of the stomach (4).
In liver, i.r. administration of both I3C and 5,6BF caused a significant induction of P-450IA mRNAs around 4 h, followed by an increase in the P-450IA1 protein level.

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P 2.18

HETEROGENOUS RESPONSE OF HEPATOCYTES TO P450 INDUCERS

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In mammalian livers differential zonal distribution and induction of cytochromes P450 have been reported. It has been suggested that the heterogeneous induction is due to variation in the sinusoidal microenvironment in different acinar zones resulting from the unidirectional perfusion of the cords of hepatocytes in the acinus. However, it has also been proposed that the differential induction of P450s within the liver acinus is due to differences in hepatocyte phenotypes. To test these hypotheses we examined, using immunochemical techniques, the inducibility and the distribution of cytochromes P450 IIB1/IIB2 and IA1 both in vivo and in vitro, using selectively isolated periportal and perivenous hepatocytes.

When rats were administered a low dose of Aroclor 1254 (ARO, 50mg/kg) or TCDD (0.25μg/kg), P450 IA1 was only induced in hepatocytes located in the perivenous region of the liver. In striking contrast, when rats were administered a low dose of β-naphthoflavone (BNF, 15mg/kg) P450 IA1 was preferentially induced in the periportal region. However, when rats were administered maximal inducing doses of ARO (1g/kg), TCDD (10μg/kg) or βNF (100mg/kg), a panacinar induction of IA1 was observed.

When rats were administered a low dose of phenobarbital (PB, 15mg/kg) or ARO (50mg/kg), P450 IIB1/IIB2 were induced primarily in hepatocytes located in the perivenous region. However, when maximal inducing doses of PB (100mg/kg) or ARO (1g/kg) were administered to rats, the induction of IIB1/IIB2 was extended to the majority of the hepatocytes situated in the periportal region.

In vitro experiments, using cultured hepatocytes isolated from the whole liver, showed a clear heterogeneous induction of IIB1/IIB2 or IA1 among cultured hepatocytes exposed to submaximal inducing concentrations of PB and βNF respectively. Furthermore there was evidence of a dose-dependent phenomenon, whereby increasing concentrations of inducer in the culture medium resulted in increasing proportions of "induced" hepatocytes. Clearly, the phenomena of heterogeneous induction of P450s and dose-dependency are retained in this in vitro system.

These studies have been extended by comparing the induction response of periportal and perivenous hepatocytes, isolated by digitonin-collagenase perfusion of the liver of untreated rats. Preliminary experiments have shown that after exposure of hepatocytes to PB, the induction of IIB1/IIB2, was far greater in the perivenous hepatocytes than in the periportal hepatocytes.

Conclusions: 1. Individual hepatocytes exhibit marked differences of sensitivity to P450 inducers. 2. As demonstrated by the in vitro studies, the heterogeneous sensitivity of the hepatocytes does not appear to be related to variation in the sinusoidal microenvironments. 3. The differential acinar induction of P450 IA1 by submaximal inducing concentrations of βNF or TCDD suggests the existence of more than one mechanism of induction (eg different receptors). 4. Primary culture of perivenous and periportal hepatocytes appears to be a useful model for studying the mechanism of induction of P450 isozymes.

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POSTERS III (P 3.1 - 3.29): TOXICOLOGY

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CHEMICALLY INDUCED LIVER INJURY AND INCREASED COUMARIN 7-HYDROXYLASE ACTIVITY IN C57BL/6 AND DBA/2 MICE

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Previous studies have established that phenobarbital and pyrazole increase considerably the activity of coumarin 7-hydroxylase (COH) in mouse liver microsomes. In connection with studies on pyrazole we found that it decreases most other P450-associated enzyme activities, apparently as a result of liver damage. Consequently, we wanted to study other liver-damaging compounds and chose first cobalt chloride and cerium, both known to cause liver injury and to decrease several P450 enzymes. Both substances (cobalt up to 40 mg/kg once or three times; cerium up to 2 mg/kg once to C57BL/6 and DBA/2 mice) caused histologically verified damage to the liver, the severity of which was dose-dependent. However, COH activity was increased between 4- and 10-fold maximally in a dose-dependent manner depending on the exact time point after the administration. Many other parameters studied, including the amount of P450 and activities of e.g. 7-ethoxyresorufin O-deethylase and 7-pentoxyresorufin O-depentylase, were slightly increased, unchanged or decreased depending on the dose of the liver-damaging agent and the time point after the pretreatment. A very serious cerium-induced liver damage led to a decrease in COH activity. We conclude that at intermediate levels of liver injury caused by pyrazole, cobalt and cerium, COH activity, in contrast to many other P450-associated activities, is considerably increased. Whether there is any causal connection between liver injury and COH increase, cannot be certainly stated, but it is worth to note that also phenobarbital and several pyrazole derivatives which do not cause at least a frank liver damage, also increase COH activity quite considerable. The mechanism of this curious phenomenon is under further investigation.

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P 32

METABOLISM AND TOXICITY OF A NEW BETA-BLOCKING AGENT (B24/76)

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B 24/76 (dl-1-(2,4-dichlorophenoxy)-3- [2-(3,4-dimethoxyphenyl) ethylamino] -propan-2-ol) is a new selective β_1-adrenoceptor antagonist with a vasodilating effect (1). The substance is metabolized in various animal species and human beings to a high extent [illegible] One of the metabolites is 2.4-dichlorophenol (2.4-DCP), a chlorophenol which is also known as an environmental pollutant with a possible toxicological importance. The aim of the study was to estimate the renal excretion of this metabolite after oral application of B 24/76 to rats and humans and to estimate the toxicological risk arising from this substance.
The betablocker was given to Wistar rats of either sex in a dosage of 100 mg/kg, which was also used in the chronic toxicity-test. In one experiment a single dose was given, in a second one the dose was repeated over 11 days. Six male volunteers received the substance orally in capsules containing 440 mg during clinical evaluation (phase I). Urine was collected at intervals of 8 - 12 h and analysed for 2.4-DCP by a GC-method, described earlier by us (3). At the end of the study animals were sacrificed and organs removed for histological and biochemical investigations.
When the substance was given to rats 5 % of the dosage were excreted as 2.4-DCP, mostly in its conjugated form. After a single application male rats showed a fourfold excretion of the free form compared with females. This difference disappeared after repeated application. In liver and kidney we found histological alterations and changes in some membrane-bounded enzymes. The amout of 2.4-DCP excreted was about two times higher than after application of a 2.4-DCP dosage at the no-effect level of 0.8 mg/kg. In contrast to rats human beings excreted only trace amounts of free 2.4-DCP and less than 1 % of the given dosage as conjugated metabolite. A toxicological relevance of 2.4-DCP for humans after administration of B 24/76 can be largely excluded on the basis of the estimated no-effect level for rats.

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P 33

NEPHROTOXICITY OF RADIOSENSITIZERS AS EVIDENCED BY WHOLE-BODY AUTORADIOGRAPHY AND ENZYMURIA

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It is well demonstrated that hypoxic cells of both animal and human tumors contribute to the relative resistance of radiation therapy. Accordingly, methods which overcome the contribution of hypoxia to radiation resistance could be expected to improve the effectiveness of radiotherapy. One of the more promising approaches involves pre-irradiation administration of drugs known as radiosensitizers.

Over the past few years, a number of substituted 2-nitro-imidazoles have been developed as radiosensitizers for the tretament of cancers. Misonidazole or 1-(2-nitro-1-imidazolyl)-3-methoxy-2-propanol (Ro 07-0582) was the first compound to be developped in this aim. Pimonidazole or 2-nitro-α-(piperidinomethyl)-1-imidazole ethanol (Ro 03-8799) is a potent, basic hypoxic cell radiosensitizer which has been shown to be up to 2-3 times as effective as Misonidazole.

With both compounds labelled with carbon 14, distribution studies have been carried out using whole-body autoradiography on normal rats and rhabdomyosarcoma-bearing rats.

These studies demonstrated that with both compounds there was a general distribution of radioactivity in the body. If we consider only the kidney, it was shown that the isotope was rather homogeneously distributed in this organ of rats dosed with Misonidazole. With Pimonidazole, it was demonstrated that in the first two hours after injection, the radioactivity in this organ was high ; then, on animals sacrificed later it was found that the isotope concentration decreased in the kidney basin and increased at the cortico-medullary junction and the medulla.

As relatioships between kidney distribution and nephrotoxicity has been found with some other xenobiotics, we started an investigation in order to find a signification to this distribution pattern of radioactivity in the kidney of rats. Rats were randomized into 3 groups. In group 1, ten animals were dosed with a single dose of 100, 250, 350 or 500 $mg.kg^{-1}$ of Ro 03-8799 and ten animals with 500 $mg.kg^{-1}$of Misonidazole. In group 2, rats were given daily 100 or 500 $mg.kg^{-1}$ of Ro 03-8799 for five days. Control animals were in group 3. All the animals were maintained in metabolic cages in order to separate urine and feces. Urine were cooled and they were collected every 24 hours. Then they were centrifuged 5 min (3000 g). Catalytic concentrations of NAG, AAP, γGT, LDH AP and concentrations of creatinine were daily measured using a commercial kit (Roche) adapted to a centrifugal analyzer (Cobas Fara, Hoffmaén Laroche).

It was demonstrated that on animals dosed once with 350 or 500 $mg.kg^{-1}$ of Ro 03-8799, a significant increase of catalytic concentrations occured 24 hours after gavage in urines for NAG, LDH and to a lower extent γGT. No modification occured on animals dosed with Misonidazole. On animals dosed five days with Pimonidazole, it is observed an increase for the same enzymes, but the enzymuria is detectable 6 days after the first gavage.

All these data are consistent with a slight nephrotoxic side action of Pimonidazole . These results illustrate the interest of distribution studies carried out by whole-body autoradiography as a potential predictive test for toxicity studies.

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METABOLIC ACTIVATION OF CHLORINATED BENZENES BY RAT LIVER MICROSOMES

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Halogenated benzenes require metabolic activation in order to cause toxicity, mainly to the liver and the kidney. For bromobenzene, epoxides have been implicated in the hepatotoxic effects, whereas the nephrotoxicity is thought to be mediated by a quinone metabolite. In contrast, tetrachlorobenzoquinone has been described as the sole reactive species formed during microsomal metabolism of hexachlorobenzene (1). We are currently investigating the microsomal metabolism of a range of chlorinated benzenes in order to establish the nature of the reactive intermediates formed.

Microsomal metabolism of 50 μM pentachlorobenzene (PECB) by DEX-microsomes (P450IIIAI) to pentachlorophenol (63%), tetrachlorophenols (18%), tetrachlorohydroquinone (3%) and metabolites covalently bound to protein (8%) has recently been described (2). Similarly, 100 μM 1,2,4-trichlorobenzene (TRCB) was metabolised to trichlorophenols (80%), trichlorohydroquinone (5%) and covalently bound material (8%), whereas 100 μM 1,4-dichlorobenzene (DICB) was metabolised to dichlorophenols (35%) and dichlorohydroquinone (26%). Protein binding of DICB metabolites was relatively high (31%). Covalent binding to exogenously added DNA was also detected, although to a much lesser extent than the protein binding. DEX-microsomes clearly showed the highest activity towards all 3 isomers tested (690%, 560% and 410% over control microsomes for PECB, TRCB, and DICB, respectively). The relative activities of microsomes from PB-, 3MC-, and ISF-treated rats towards the 3 chlorinated benzenes varied considerably: whereas 3MC-microsomes hardly metabolised PECB, they showed considerable activity towards the lower chlorinated benzene derivatives.

The covalent interaction with protein was reduced to a great extent by the addition of 1 mM ascorbic acid, a strong reducing agent. A concomittant increase in the amount of chlorinated hydroquinone metabolites occured, indicating an important role for quinone metabolites in the protein binding of all 3 chlorinated benzenes. DNA-binding in the presence of ascorbic acid was only reduced by 60%. Our results indicate that the formation of quinone metabolites during the microsomal conversion of chlorinated benzenes is the major route of metabolic activation, although epoxides may play a (minor) role in the observed DNA-binding.

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P-450 IA2 AND DRUG INDUCED HEPATITIS.

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Sera from patients with dihydralazine induced hepatitis were shown to contain anti-liver microsomal antibodies (anti-LM) by indirect immunofluorescence. These anti-LM antibodies were different from anti-Liver/kidney microsomes (anti-LKM) 1 or 2 autoantibodies which have been previously described. They recognised specifically by immunoblotting in the microsomes from human liver a protein with a Mr of 53 000. This protein was identified as cytochrome P-450 IA2 by several studies. As P-450 IA2 is inducible by β-naphtoflavone and isosafrole. induction studies were performed in rats by dihydralazine (150 mg/Kg i.p. daily for 3 days). An induction of P-450 IA2 and IA1 was observed by immunoblot and. this finding is confirmed by an significant increase of ethoxyresorufin O-deethylase activity (supported by both P-450 IA1 and P-450 IA2) and methoxyresorufin O-dealkylase activity supported mainly by P-450 IA2.

Dihydralazine competitively inhibited the ethoxyresorufin O-deethylation in human liver microsomes suggesting that P-450 IA2 might metabolize dihydralazine.

In order to further evaluate the role of P-450 IA2 in Dihydralazine metabolism and the first steps triggering the disease. we investigated long term effect of Dihydralazine treatment in rat.

This is the third example of autoimmune hepatitis in which an isoenzyme of Cytochrome P-450 was the specific target of the auto-antibodies.

The interaction between Dihydralazine and P-450 IA2. (metabolism. induction. mechanism of disease triggering) will be discussed.

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THIOCOMPOUNDS AS POSSIBLE PHOTOPROTECTIVE AGENTS.

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Possible (sun)light-protective properties of some thiols were investigated. Captopril (CAP), Cysteamine, D-penicillamine, Mercaptopropionylglycine (MPG), Mesna and N-acetylcysteine (NAC) are, or have been, used as a therapeutic for a variety of disorders. Ergothioneine is a thiol with possible importance in fysiological processes of living organisms.

As a measure for protection, inhibition of irreversible photobinding of chlorpromazine (CPZ) and 8-methoxypsoralen (8-MOP), to DNA and protein (HSA) was used. (Photobinding of CPZ proceeds via promazinyl-radicals (PZ•); with 8-MOP this occurs via radicals and cycloaddition). Solutions of 8-MOP (or CPZ), DNA (or HSA) and thiols in water (pH=7.4) were UVA-exposed. With CPZ, besides photobinding, the photodegradation of CPZ itself and the formation of the two only photoproducts promazine (PZH) and hydroxypromazine (PZOH) were measured as well. (PZH and PZOH result from reaction of PZ• with thiols and with H_2O. Because of the H-atom and electron donating capacity of thiols, both the ratio [PZH]/[PZOH] and the photodegradation of CPZ was expected to be higher in the presence of thiols. The latter by direct reaction of thiol with photo-excited CPZ).

Maximum inhibition of photobinding of CPZ ranged for the different thiols between 50-87% (HSA) and 74-99% (DNA). All thiols enhanced the photodegradation of CPZ, decreased PZOH production and markedly increased PZH formation. 8-MOP photobinding to HSA was also clearly inhibited (66-89%), but, remarkably, hardly that to DNA (2-26%).

In vivo results, with rats treated topically with 8-MOP (77 nmol/cm²) and NAC, MPG or CAP (2 umol/cm²), prior to UVA-exposure, showed marked inhibition of photobinding to lipids (59-79%) and proteins (39-60%), but unexpectedly also to DNA/RNA (60-77%). Vitamin-E and BHT, extensively investigated because of their possible sunprotecting activity gave under comparable conditions the following results. Inhibition of photobinding to lipids, proteins and DNA/RNA: 0%, 21% and 18% for vitamin-E and 12%, 14% and 8% for BHT. Topical treatment with CPZ (77 nmol/cm²) and NAC (10 umol/cm²) gave an inhibition of photobinding to lipids with 83% and to proteins with 70%. (Photobinding of CPZ to DNA in vivo, without thiol, was below detection limit). Remarkably, vitamin-E and BHT did not inhibit CPZ photobinding at all, neither to proteins nor to lipids.

From these results the conclusion seems justified that thiols are more potent sunprotecting agents than vitamin-E and BHT.

2028903658

EFFECT OF CYTOCHROME P450 INDUCTION ON MUTAGENICITY OF 2-AMINOANTHRACENE IN LIVER AND INTESTINE OF RATS.

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Many environmental chemicals have been shown to cause cancer in experimental animals. Most of these compounds are innocuous by themselves and need biotransformation into ultimate carcinogens or mutagens by xenobiotic metabolizing enzymes. The cytochrome P450 family plays an important role in mutagen activation and xenobiotic toxication. The cytochrome P450 enzymes have various and overlapping substrate specificity and inducers. Their induction may modify the balance between detoxication and toxication pathways, and consequently their mutagenic capacity. Indeed, pretreatment with pregnenolone-16-α carbonitrile (a P450 IIIA subfamily inducer), significantly reduce the incidence of liver carcinomas in rats exposed to dimethylnitrosamine (metabolized by P 450 IIE isoenzyme). Moreover, the alimentary tract constitutes the primary exposed tissue to ingested xenobiotics and it is interesting to study the effect of intestinal cytochrome P 450 induction on mutagenic activation. The mutagenicity of 2-aminoanthracene (2AA), a test compound metabolized by P450 IA enzymes, was studied in the intestine, colon and liver of induced or non-induced rats.

Thus , male Sprague-Dawley rats, weighing 100-200 g, were treated by different inducers: Phenobarbital (PB) was given at a dose of 0.1% in drinking water for 10 days; the other inducers were administered intragastrically, daily for 3 days: ß-naphthoflavone (BNF) 40 mg/kg, dexamethasone (DX) 50 mg/kg, acetone (AT) 50%, dexamethasone and acetone (DX + AT). The post-mitochondrial supernatants (S9) of liver, duodenum, jejunum, ileum and colon were used for in vitro studies and the mutagenic activation of 2AA was detected in the *Salmonella typhimurium* mutagenic assay (Ames test).

In all tissues investigated, BNF induction increased considerably the mutagenic potency of 2AA (3 to 4 fold) whereas aroclor induction lowered the mutagenicity of 2AA in the liver. By contrast, the other treatments: PB, DX, AT and DX+AT significantly reduced the mutagenicity of 2AA, in all these tissues.

Our results showed that Aroclor, currently used as inducer to study the mutagenic potency of numerous xenobiotics in Ames tests, could lead to an undervaluation of the actual mutagenicity of certain compounds. Moreover specific inducers of P 450 isoenzymes can modulate positively or negatively the mutagenic activation of 2AA and likely of some other xenobiotics in several tissues.

Variations of mutagenic activation will be related to P 450 and glutathione transferases expression in every studied tissues.

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HALOTHANE HEPATOTOXICITY: EVIDENCE FOR TRIFLUOROACETYLATED POLYPEPTIDES IN KUPFFER CELLS

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Fulminant massive liver necrosis ("halothane hepatitis") is the more severe form of hepatotoxicity associated with the anesthetic agent halothane. Upon single exposure to the anesthetic, ~ 1 in 10000 patients is afflicted. There is ample evidence suggesting that halothane hepatitis has an immunological basis. Most convincingly, sera of halothane hepatitis patients, but not those of according control individuals, contain antibodies against prominent liver heterologous microsomal polypeptides (so-called TFA-adducts), triflouroacetylated upon oxidative, cytochrome P450-dependent metabolism of halothane. TFA-adducts have previously been detected immunochemically on the surface of isolated rat hepatocytes. Mechanisms by which, in hepatocytes, TFA-adducts could be presented to competent cells of the immune system and serve as potential neo-immunogens remain to be elucidated.

We have speculated that liver resident macrophages (i.e. Kupffer cells), naturally competent for antigen presentation, might be involved in presenting TFA-adducts to cells of the immune system. Therefore, we have isolated, from phenobarbital induced male Sprague Dawley rats, pretreated with a single dose (10 mmol/kg body weight) of halothane 18h before sacrifice, through in situ collagen/dispase perfusion, Percoll gradient enrichment and selective plastic adherence techniques, hepatocytes and Kupffer cells, which are essentially free of contaminating cells. Using a rabbit polyclonal anti-TFA-antibody, affinity-purified on a N-ε-TFA-lysine coupled Affi-Gel 102 matrix, we probed, after separating polypeptides on SDS-PAGE and transfer to nitrocellulose, homogenates of these cells for the occurrence of TFA-adducts on Western blots. Both hepatocytes and Kupffer cells from halothane treated, but not control animals, exhibited reactivity of the anti-TFA-antibody towards a number of target polypeptides; binding could be reversed through competition with N-ε-TFA-lysine or cleavage of the TFA-group from modified polypeptides by piperidine. The apparent molecular masses (M_r) of the most prominent TFA-adducts recognized in hepatocytes were 100000, 78000, 59000, 55000, 50000, and 45000; several minor TFA-adducts of distinct M_r were detected as well. Except for the M_r 50000 polypeptide, TFA-adducts were persistent and could be detected 96h after a single halothane dosis; phenobarbital induction was not a prerequisit for extensive TFA-adduct formation. The M_r of TFA-modified polypeptides recognized in homogenates of Kupffer cells 18h after halothane were identical to those observed in hepatocytes. Our findings suggest that Kupffer cells may be involved in neo-immunogen processing and presenting; according experiments are in progress.

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ROLE OF THE BALANCE OF DEACETYLATION AND REACETYLATION IN RELATIVE NEPHROTOXICITY OF MERCAPTURIC ACIDS OF FLUOROETHYLENES IN RATS.

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Mercapturic acids (N-acetylcysteine S-conjugates, NAc) were previously shown to be suitable model compounds to study cysteine conjugate ß-lyase-mediated nephrotoxicity of halogenated alkenes in the rat [1]. Intracellular N-deacetylation of the mercapturic acids by renal acylases provides cysteine conjugate to the activating enzyme ß-lyase in the proximal tubular cell. (Re)acetylation of the cysteine conjugate, catalyzed by microsomal N-acetyltransferase, however, antagonizes the initial activation step (N-deacetylation), and may therefore be regarded as a detoxication step.

In this study, the relationship between the relative nephrotoxicity of the mercapturic acids of the fluorinated ethylenes tetrafluoroethylene (TFE), chlorotrifluoroethylene (CTFE), 1,1-dichloro-2,2-difluoroethylene (DCDFE) and 1,1-dibromo-2,2-difluoroethylene (DBDFE), and the biotransformation by activating (N-deacetylase and ß-lyase) and inactivating (N-acetyltransferase) enzymes was studied in the rat.

The threshold dose of the mercapturic acids to cause nephrotoxicity in male Wistar rats increases in the order: CTFE-NAc (25 µmol/kg) < TFE-NAc (50 µmol/kg) < DCDFE-NAc (75 µmol/kg) < DBDFE-NAc (100 µmol/kg). By administrating N-(trideuteroacetyl)-labeled mercapturic acids to rats, it is demonstrated that the less toxic conjugates DCDFE-NAc and DBDFE-NAc are excreted in urine unchanged (e.g. still labeled) at much higher amounts (30-50% (of the dose)) than the more toxic conjugates TFE-NAC and CTFE-NAc (1-2%). Also, the presence of high amounts (45-54%) of unlabeled DCDFE-NAc and DBDFE-NAc points to an efficient reacetylation of the cysteine conjugates formed. With the more toxic conjugates TFE-NAc and CTFE-NAc reacetylation only is a minor route (<2%). Additional in vitro metabolism data indicate that higher rates of N-deacetylation and, to a minor extend, ß-lyase activation may determine the higher nephrotoxicity of TFE- and CTFE-derived mercapturic acids.

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SODIUM NITRITE-STIMULATED METABOLIC ACTIVATION OF BENZO(A)PYRENE-7,8-DIHYDRODIOL IN HUMAN POLYMORPHONUCLEAR LEUKOCYTES

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Introduction: Benzo(a)pyrene-7,8-dihydrodiol (BP-7,8-diol) is a metabolite of benzo(a)pyrene (BP), a widely distributed environmental pollutant and a probable factor in the development of lung cancer. BP-7,8-diol can undergo epoxidation to the reactive intermediate BP-7,8-diol-9,10-epoxide which is supposed to be the ultimate carcinogenic metabolite of BP. Nitrogen oxides (NO_2, NO) are environmental pollutants which may be cocarcinogenic with polycyclic aromatic hydrocarbons (PAH), by affecting their metabolism and activation in different ways. NO_2 dissolves readily into mucous and into epithelial cells throughout the lung and is rapidly distributed in the body via the pulmonary circulation. The major chemical intermediates of inhaled NO and NO_2, detectable in blood and urine, are nitrite (NO_2^-) and nitrate (NO_3^-), and many of the toxicological effects of NO and NO_2, including cocarcinogenicity with PAHs, are possibly mediated via these metabolites, particularly NO_2^-. Nitrite itself has a number of detrimental effects on physiological functions including the conversion of hemoglobin to methemoglobin, the ability to ligate to the iron of different heme proteins and to act as a single electron donor in oxidation reactions involving catalase, peroxidases and hemoglobin.

Results and Discussion: Sodium nitrite stimulated the metabolism of BP-7,8-diol in phorbol ester-activated polymorphonuclear leukocytes (PMNs). The products of BP-7,8-diol metabolism measured in our experiments included the stable tetrols (7/8,9,10- and 7,10/8,9-tetrahydrotetrol) and tissue-bound species. Both tetrol production and the covalent binding to exogenous DNA and cellular proteins were significantly ($p<0.05$) enhanced in the presence of sodium nitrite. This stimulatory effect was only observed in the presence of the phorbol ester, phorbol myristate acetate (PMA), which induced oxygen activation in the PMNs. The sodium nitrite-dependent activation of BP-7,8-diol in PMA-stimulated PMNs appears to be peroxidase-dependent, probably mediated by myeloperoxidase (MPO), since both tetrol production and tissue binding were inhibited by sodium azide.

Conclusions: In summary we have shown that sodium nitrite can stimulate the metabolism of BP-7,8-diol to reactive intermediates which can covalently bind to DNA and proteins. The reactions seem to be peroxidase-dependent and catalyzed by MPO in PMA-stimulated PMNs. These findings may be of significance in terms of the potential cocarcinogenic effects of NO_2 and nitrite with PAHs.

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BIOCHEMICAL CHARACTERIZATION OF NOVEL ANIONIC GLUTATHIONE S-TRANSFERASES FROM RAT LIVER AND THEIR TOXICOLOGICAL RELEVANCE IN THE METABOLISM OF XENOBIOTICS

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Glutathione S-transferases (GST;E.C. 2.5.1.18) are a family of enzymes which can be separated into three classes (alpha, mu and pi) on the basis of structural, immunological and catalytic properties. The finding that the acidic isoenzymes of the µ-class possess a great potential in conjugating diolepoxides of polycyclic aromatic hydrocarbons (PAH) attracted increasing interest in the elucidation of their toxicological role.

A very efficient purification procedure for acidic GST isoenzymes was developed including anion exchange chromatography on QA-cellulose, affinity chromatography on GSH-sepharose as well as chromatofocussing on Mono P (FPLC) and hydroxylapatite (HTP)chromatography. Chromatofocussing in the pH range 7-5 revealed six major GST fractions (GST I-VI) between pI 6.8 and 5.3, which could be further separated into a total of 12 forms (GST-HTP 1-12) by HTP chromatography of every single fraction (I-VI). On SDS-PAGE the subunits of GST-HTP 1-12 focussed between 24-27 kD. Subsequent 2D-gel electrophoresis and HPLC studies on a Dynamax 300 Å column pointed out, that at least 9 of these GST forms represented pure homo- or heterodimers. These isoenzymes were further characterized by IEF, substrate specificities with diagnostic substrates and immunological studies with polyclonal antibodies against the prominent and well-characterized rat liver GSTs. Except for GST-HTP 3, which is obviously similar to, if not identical with the recently discovered and well studied GST 8-8, and two immunologically related forms, all dimers crossreacted with antibodies directed against the µ-class isoenzyme GST 4-4, which is known to exhibit high turnover rates with mutagenic and carcinogenic PAHs. In Ames Tests with the mutagenic compounds BA-5,6-oxide and BA-8,9-diol-10,11-oxide all GST isoenzymes described in this work showed significant but very different activities with the former substrate, GST-HTP 5 giving a 50% inactivation with as little as 3ng/plate. The latter substrate was conjugated by at least six isoenzymes (GST-HTP 2,4,5,7,10 and 11) at much lower rates. These results suggest a remarkable role of these novel anionic isoforms in the control of genotoxic metabolites.

Results with monoclonal antibodies, which were raised against the purified acidic isoenzymes and shown to be capable of distinguishing between those, are presented.

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SULFOTRANSFERASE-MEDIATED HALOGENATION OF BENZYLIC ALCOHOLS TO MUTAGENS

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Methyl-substituted polycyclic aromatic hydrocarbons are common in the human environment. Many of them are stronger carcinogens than their non-alkylated aromatic congeners. They may be metabolically hydroxylated to benzylic alcohols. 1-Hydroxymethylpyrene (HMP) and 9-hydroxymethylanthracene (HMA), representatives of this class of compounds, were activated to mutagens, detected in *Salmonella typhimurium* TA98, in the presence of rat liver cytosol (or purified hydroxysteroid sulfotransferase) and 3'-phosphoadenosine-5'-phosphosulfate (PAPS). This mutagenicity was weak when the exposure took place in the absence of halogen anions. It was potent when Cl^- was added in physiological concentration. The authentic sulfate esters of HMA and HMP proved to be mutagenic in the absence of mammalian enzymes. However, their mutagenic potency was increased about 50 times when Cl^- was added in physiological concentration. A similar effect was observed in the presence of Br^-. In chloride-free and chloride-containing physiological buffer at 37 °C, HMP sulfate had a half-life of 2 min, the major decomposition product being HMP. Thus, cyclic activation is possible. When Cl^- was present at physiological concentration, an additional reaction product, 1-chloromethylpyrene (ClMP), could be identified on the basis of its chromatographic behaviour and its mass spectrum, using the authentic standard for comparison. ClMP was shorter-lived in buffer than HMP sulfate. It was an extremely potent mutagen.

When incubated with free DNA, both HMP sulfate and ClMP formed adducts. The adduct patterns in the ^{32}P-postlabelling analysis were similar, or identical, with both compounds. Cl^- ions showed no appreciable effect on quantity or quality of the adducts formed from either compound. It is therefore concluded that HMP sulfate is capable of reacting with DNA, but is hampered in its distribution by membrane barriers. Strikingly, a short-lived ClMP intermediate is produced, which can act as transport form to overcome membrane barriers. It is important to know that HMP- and HMA-activating sulfotransferase are distributed very unequally in the organism. For example, the activity in parenchymal liver cells exceeded that in Kupffer cells by a factor of ≥ 200. Distribution processes and their restriction may, therefore, be important factors determining the toxicology of benzylic alcohols and other compounds activated through conjugation with sulfate.

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ACTION OF MECLOFENOXATE, THEIR METABOLITES, AND PIRACETAM ON THE ISOPROTERENOL-INDUCED PROCESSES IN THE RAT HEART.

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Meclofenoxate and piracetam show a reduction of accumulated lipofuscin, enhance the rates of the RNA synthesis and of the glucose uptake with a shift from glycolysis to the pentose phosphate pathway. Several authors have tested these drugs, socalled nootropics, with respect to its protective action, but the results are controversial, mainly caused by their different experimental techniques. Therefore the following questions are of interest:

1. Show the substances any cardioprotective effect?
2. Is there a remission of the isoproterenol-induced heart hypertrophy?
3. Are the effects observed related to the intact substances or to their metabolites?

All experiments were performed in rats, pretreated with 5 mg/kg of isoproterenol given daily for ten successive days to induce heart cell necrosis and heart hypertropy. Meclofenoxate, their metabolites (p-chlorophenoxyacetic acid and dimethylaminoethanol), and piracetam were given daily in doses from 50-100 mg/kg for ten consecutive days to prevent the isoproterenol-induced effects on the heart. The heart mass development, the activity of dehydrogenases and of the ornithine decarboxylase, the myocardial content of RNA, the energy state and the myosin isoenzyme v1,v2 and v3 were investigated.

All drugs tested in this model are unable to prevent the heart hypertrophy induced by the isoproterenol. Under the influence of the isoproterenol the v3 myosin increases at the expense of the other two, especially the v1 which disappeared completely. In combined application there is a shift to the redistribution mediated by the nootropics and these metabolites. Meclofenoxate, p-chlorophenoxyacetic acid, dimethylaminoethanol, and piracetam show characteristic cardioprotective effects on the myocardium. This was indicated by a reduced mortality of the animals and a reduced loss of the activity of the mitochondrial NADH-nitro-BT-reductase. The decreased content of the RNA and of the reduced energy state produced by the isoproterenol were gradually normalized by the nootropics. Therefore we conclude, that the nootropics pirazetam and meclofenoxate as well as the metabolites of the latter have a cardioprotective action.

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ACETYLATOR GENOTYPE-DEPENDENT N-ACETYLATION OF ARYLAMINE CARCINOGENS IN HUMAN COLON CYTOSOL FROM CANCER-FREE AND COLORECTAL CANCER PATIENTS

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Two human epidemiological reports have suggested an association between the rapid acetylator phenotype and risk of colorectal cancer. Recent unpublished studies have not found this association. The hypothesis for an association between acetylator phenotype and colorectal cancer suggests an acetylator genotype-dependent expression of arylamine N-acetylation capacity in the human colon, catalyzed by acetyl coenzyme A-dependent N-acetyltransferase(s) (EC 2.3.1.5) (NAT). Human colon cytosols from forty-eight fresh surgical samples were investigated for NAT activity towards p-aminobenzoic acid and the arylamine carcinogens 4-aminobiphenyl, 2-aminofluorene, and β-naphthylamine. Apparent Vmax determinations of NAT activity towards these substrates indicated that forty individual colons segregated into three distinct phenotypes. The distribution of the patients into rapid (5), intermediate (18), or slow (17) acetylators is a ratio that is not significantly different from the expected Hardy-Weinberg distribution of 3:16:21 (X^2 = 2.206, p = 0.363). Significantly greater mean apparent Vmax levels were found in colons from rapid as compared to intermediate acetylators (1.5-3 fold) ($p<0.001$) and intermediate compared to slow (2.5-3 fold) ($p<0.005$) acetylator phenotypes for the four arylamine substrates. The corresponding apparent Km determinations indicated that human colon NAT from rapid acetylators showed significantly lower affinity for the arylamine substrates ($p<0.05$) compared to intermediate or slow acetylators. No difference in apparent Km was detected for the cofactor acetyl coenzyme A between the three acetylator phenotypes. The colon cytosols were also tested for their capacity to activate N-hydroxy-2-aminofluorene (N-OH-AF) to a DNA binding electrophile through a direct O-acetyltransferase (OAT)-mediated catalysis. Although kinetic studies were not done, the human colon cytosols showed no significant differences in OAT-mediated DNA binding of N-OH-AF at the substrate concentration selected (100 μM N-OH-AF). Of the forty colon samples, thirty-seven were from individuals diagnosed with colorectal cancer (twenty-five) or cancer-free (twelve). No significant differences between the cancer-free and colorectal patients were observed in mean apparent Km and Vmax levels for NAT activity in the three acetylator phenotypes. However, a greater proportion of slow acetylators was found in the colorectal patients (52%) as compared to the cancer-free patients (25%). Partially supported by USPHS grants CA-34627 and RR-08248 from the National Cancer Institute, USA.

2028903662

BIOACTIVATION OF QUINONE CYTOSTATICS BY CYTOCHROME P450

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Chemotherapeutic quinones such as mitomycin and adriamycin require reductive metabolism to exert their cytostatic effect. The reduction of such quinones (Q) to semiquinone radicals ($SQ^{\cdot}$) can be catalyzed by NADPH-cytochrome P450 reductase (RED; 1). One-electron reduction reactions can also be catalyzed by cytochrome P450 (P450; 2). Since the abundance of P450 in the liver cell is 20 times higher than that of RED (3), the hypothesis that P450 might play an important role in the bioactivation of quinone cytostatics was investigated with duroquinone (DQ; 2,3,5,6-tetramethyl-1,4-benzoquinone, a simple model quinone), and adriamycin (ADR).

The contribution of P450 and RED in the reduction of DQ and ADR was assessed in two ways. First, the effect of inhibitors of P450 on the microsomal metabolism of DQ and ADR was investigated. Second, metabolism was studied with purified rat liver enzymes in reconstituted systems consisting of RED only or of equimolar amounts of RED and P450-IIB1. Metabolism of quinones was determined by quantitation of $SQ^{\cdot}$ formation (with Electron Spin Resonance, ESR) and hydrogen peroxide (H_2O_2) formation under anaerobic and aerobic conditions, respectively.

Under *anaerobic* conditions, $SQ^{\cdot}$ radicals of DQ and ADR could be easily detected in microsomes from phenobarbital-treated rats. SKF-525A reduced $SQ^{\cdot}$ formation to about one third (for DQ and ADR). In reconstituted systems with DQ as substrate, P450-IIB1 in the presence of RED was 4 times more active in $SQ^{\cdot}$ formation than RED alone. With ADR the fully reconstituted system (P450-IIB1 plus RED) was two fold more active in $SQ^{\cdot}$ formation than the reconstituted system with RED only.

As expected, under *aerobic* conditions (air-saturated buffer), $SQ^{\cdot}$ formation could hardly be detected due to redox cycling. In accordance, superoxide anion could be detected by ESR with the spin trap DMPO. Further, H_2O_2 the dismutation product of superoxide anion was formed. In agreement with the findings under anaerobic conditions, SKF-525A decreased quinone-induced H_2O_2 formation to about one third (for DQ and ADR).

In conclusion, the present data support the hypothesis that P450 may contribute substantially to the bioactivation of quinone cytostatics. At present the hypothesis is studied by chemical reduction of P450.

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PRIMARY RENAL CELL CULTURE AS A TOOL FOR IN VITRO TOXICITY EVALUATION

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Ochratoxin A is produsued by several species of Aspergillus Ochraceus and Penicillum. It is a potent nephrotoxin and has been observed as a major factor of porcine nephropathy /Krogh 1978/. The aim of this study is to trase the effect of various doses of Ochratoxin A on a primary culture system of rat kidney cortical cells to evaluate the nephrotoxicity of xenobiotic. The action of the toxin on the growth of cells and their morphological and methabolic characteristics was studied. A primary culture system of postnatal rat kidney cortical cells was made by modifying the Smith and Acosta's method /1986/.The toxin was added to the ceell cultivation media to obtain final concentrations of 2.0, 0.1, 0.01, 0.001 mkg/ml. Cells were examined to find out the cell growth rate, morphological characteristics after Giemsa, Haematoxylin - eosin, Van Gieson, Trypan blue staining and standard processing for transmission electron microscopy. Besides alkaline phosphatase and γ-glutamyltranspeptidase activity and cytochrom P-450 contents were examined. The Ochratoxin A effect was studied on the 6th, 24th, 48th, 72th, and 96th, hour.

The low Ochratoxin A concentrations seem not to affect the cells on hour 24th. Even stabilisation of the monolayer is noted. The cells are with better vitality. Upon treatment of 0.1 some cells are increased in number, even giant, some mitoses are also found. Breaking of the monolayer, degenerative changes of cytoplasm, pycnotic nuclei are found with the 2 mkg/ml Ochratoxin A concentration on hour 96, only the 0.001 doesn't destroy significantly the cells. By adding nicotinamide long-term maintenance of cytochrom P-450 in vitro is obtained. The contents of the cytochrom are increased. The effect is most pronounced on the 6th hour. The data concerning metabolic characteristics are debatable.

TRICHLOROETHYLENE ACUTE INHALATION IN MICE: UPTAKE AND CLEARANCE RATES.

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At the ENEA Research Center a program, on the biological effects caused by acute nose-only inhalation of organic compounds in rodents, is carrying out. In the present study the uptake and clearance rates of trichloroethylene (TCE) administred to BC3F1 male mice are reported.

Our previous studies showed that the exposure to different doses, ranging from 500 to 7000 ppm of TCE, selectively damages the metabolic cells (Clara) of the lung. Severity of the injury was dose-dependent and damage was maximum 24 h after the treatment.

The TCE kinetic behaviour in the blood, brain and urines of mice is determined by head-space gas chromatography. Animals were exposed to a single inhalation of 4000 ppm TCE for 30' min. In order to follow the uptake, mice were killed after 15, 30, 60 and 120 min from the beginning of the exposure. A close relationship has been found between TCE in the arterial blood and exposure times ($r > .91$). TCE uptake in the brain was also time-dependent ($r > .96$). For clearance determinations animals were killed after 0, 30, 60 and 90 min from the end of treatment. Arterial TCE levels decreased very quickly. TCE half-time was around 30 min. Urinary contents of the TCE metabolite trichloroacetic acid (TCA) has been determined 15, 24, 48, 72 and 96 h after the end of the exposure. The maximal rate of excretion was found at 24 h. TCA was evaluated by thermic decarboxylation to chloroform.

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BIODISPOSITION OF 2-2' CHLOROETHYL SULFIDE IN THE RAT AFTER IV INTOXICATION.

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The purpose of this poster is to describe the first toxicokinetic of 2-2' chloroethyl sulfide, (sulfur mustard), in rats.

Sulfur mustard is a highly toxic compound for which very few data on biodisposition are available. Therefore it is of great importance for therapeutics purposes to investigate the kinetic of this compound.

10mg/kg/ml, three time the DL50, of this compound was administred to the rat by intravenous route.

The concentrations of inchanged sulfur mustard in the blood were determined by CG-FID detection after liquid-liquid extraction. This method allowed specific determination of sulfur mustard with 83 +/. 4,4% extraction efficiencies and with reproducibility in the range of 6%.

We obtained two phases with a $t1/2\alpha$ = 5 mn and with a $t1/2\beta$ = 380 mn.

14C sulfur mustard was used to determined urinary and faecal excretion after IV intoxication.

80% of the total radioactivity was recovered in the urine after 24 hours and less than 3% in the feces.

Six metabolites have been separated from urine.

Urine, feces 0-3, 3-6, 6-12, 12-24, 24-48 etc
1,5 4,5 9 18 36 60 84 h

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202890366A

GLUTATHIONE DEPENDENT DETOXICATION IN ADULT RAT HEPATOCYTES UNDER VARIOUS CULTURE CONDITIONS.

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In previous work, the effects of various culture and medium conditions on the glutathione S-transferase (GST) activity and isoenzyme pattern have been studied in conventional cultures and co-cultures of adult rat hepatocytes and significant changes have been observed (1-4).

In order now to obtain more information concerning the effects of the same culture and medium conditions on other glutathione dependent detoxication systems, reduced (GSH) and oxidized (GSSG) glutathione contents, glutathione reductase (GR) and glutathione peroxidase GPx) activities were studied in both conventionally and co-cultured rat hepatocytes. The addition of 10% FCS, 25 mM nicotinamide, 0,1 µM selenium and 2% dimethylsulphoxide respectively to the culture medium was examined.

As far as the glutathione level is concerned, it was found that, after a pronounced initial increase, the intracellular GSH levels decreased in both culture systems. In conventional culture no stable situation was obtained, whereas in co-culture levels comparable with those obtained in freshly isolated hepatocytes were quickly reached and maintained for at least 2 weeks.

The intracellular GSSG levels were very low.

Suplementation of the culture medium with the products mentioned had no effect on the GSH and GSSG contents of the hepatocytes in both culture systems.

As far as GR and GPx activities are concerned, after an initial increase, they decreased slowly and increased again at the end of the culture period. No effect was observed by the various supplements to the medium. These results still need to be confirmed in co-cultures of hepatocytes.

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This study was supported by the NFWO (Fund for Medical Scientific Research).

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A MECHANISM FOR TRICHLOROETHYLENE INDUCED LUNG TUMOURS IN THE MOUSE.

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Trichloroethylene (TRI) is a commonly used industrial solvent. Inhalation exposure of mice to TRI has been shown to induce lung adenomas and adenocarcinomas (Fukuda 1983, Maltoni 1984). TRI administered as a single i.p dose also caused vacuolation and necrosis of the mouse lung Clara cell specifically (Forkert 1985). In the present study the mechanisms of TRI induced lung injury and its relevance to carcinogenicity have been investigated. Female CD1 mice were exposed to TRI and its metabolites by inhalation and Clara cells isolated from them. Xenobiotic metabolizing enzyme activities: ethoxycoumarin O-deethylase (ECOD), aldrin epoxidation (AO) and chlorodinitrobenzene-glutathione transferase (GT) were determined in cell sonicates. NADPH cytochrome c reductase (CR) was also measured as an indicator of Clara cell toxicity. Female Alpk: ApfSD rats were also exposed to TRI, lung microsomes prepared and the same enzyme activities measured. Mice exposed to TRI (200-2000ppm for 6h) developed Clara cell vacuolation with pyknosis and focal loss of the bronchiolar epithelium occurring at the highest doses. Enzyme activities were reduced: ECOD (55-25% control), AO (80-63% control) and CR (35-55% control) with only GT unchanged. A clear species difference was observed in the effect of TRI on rat lung morphology. The lungs of rats exposed to TRI (500 or 1000ppm for 6h) showed no pathological effects. ECOD and GT were unchanged although decreases were seen in AO (50-70% control) and CR (75% control). The biochemical effects in the mouse may result from both Clara cell toxicity and the known inhibition of P450 by TRI whereas in the rat only the inhibitory effect appears to be occurring. The metabolism of TRI in Clara cells isolated from control mice was investigated and chloral found to be the major metabolite. Trichloroethanol (TCE) and low levels of trichloroacetic acid (TCA) were also formed, but not TCE-glucuronide. In hepatocytes TCE was the major metabolite with TCE-glucuronide also readily formed. Comparison of UDP-glucuronosyl transferase activity in Clara cell sonicates and liver fractions revealed that although activity towards 4-nitrophenol and 2-aminophenol was at least equivalent to that in liver, TCE glucuronidation in Clara cells was 12 times lower than in liver. Mice exposed to chloral (100ppm for 6h) developed severe Clara cell lesions and CR was reduced (70% control). TCE (100ppm for 6h, or 500ppm for 2h) had no effect on mouse lung pathology or biochemistry. In conclusion, the rapid formation of chloral in the mouse Clara cell together with a deficiency in the metabolic pathways following this is believed to result in accumulation of chloral. The toxicity of chloral to the Clara cell together with its known mutagenicity in Ames tests is thought to be responsible for the induction of lung tumours in the mouse by TRI.

2028903665

THE ROLE OF DIHYDRODIOL AND TETRAHYDROTETROL EPOXIDES IN THE STEREOSELECTIVE METABOLIC ACTIVATION OF trans-DIHYDRODIOLS OF DIBENZ(a,h)ANTHRACENE TO BACTERIAL MUTAGENS

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Dibenz[a,h]anthracene (DBA), a carcinogenic polycyclic aromatic hydrocarbon ubiquitously present in the environment, is metabolized by the hepatic microsomal fraction of immature Sprague-Dawley rats pretreated with Aroclor 1254 to 27 ethyl acetate-extractable metabolites. More than half of their amount (51.0 %) consisted of trans-1,2-, -3,4- and -5,6-dihydrodiol (1,2-, 3,4-, 5,6-diol) including their identified secondary metabolites. The three trans-dihydrodiols (4.9, 15.8, 0.6 % of total metabolic conversion) were highly enriched in their R,R enantiomers (85, 71, 98 %) as determined by high-performance liquid chromatography on suitable chiral stationary phases. This is explained on the basis of the stereoselective epoxidation of DBA by cytochrome P450IA1 (induced by Aroclor 1254) followed by regioselective hydration catalyzed by microsomal epoxide hydrolase.

Determination of the bacterial mutagenicity by measuring the reversion of histidine-dependent Salmonella typhimurium TA100 to histidine prototrophy revealed marked differences in the mutagenicity of the enantiomers of the trans-dihydrodiols of DBA when activated by the same metabolizing system as used in the metabolism studies. In the case of 1,2- and 5,6-diol the S,S enantiomers were converted to stronger mutagenic metabolites than their corresponding optical antipodes, whereas in the case of 3,4-diol it was the R,R enantiomer.

In order to identify the ultimate mutagens of the trans-dihydrodiols of DBA and consequently of DBA itself the secondary metabolism of racemic 1,2-, 3,4- and 5,6-diol was investigated.

Principal metabolites of 1,2-diol are tetrahydro(1,3/2,4)- and -(1,4/2,3)tetrols originating from the syn- and anti-isomers of 1,2-diol-3,4-epoxide.

The surprisingly high bacterial mutagenicity of 5,6-diol has recently been explained by the possible metabolic formation of 3,4,12,13-tetrahydrotetrol-1,2-epoxide. However this pathway could not be confirmed, since the precursor of the proposed tetrahydrotetrol epoxide, 3,4,12,13-bisdihydrodiol, although metabolically formed from 5,6-diol, failed to become activated to a mutagen under metabolic conditions where the 5,6-diol was converted to strong bacterial mutagens.

The controversial discussion on the existence of tetrahydrotetrols as an indication of the formation of dihydrodiol epoxides from 3,4-diol was finally solved by the direct spectral and chromatographic identification of the chemically rather stable syn- and anti-isomers of 3,4-diol-1,2-epoxide as microsomal metabolites of 3,4-diol.

Furthermore, the identification of 3,4,10,11-bisdihydrodiol, convertible to strong bacterial mutagens, as the principal metabolite of 3,4-diol, indicates that not only the long postulated dihydrodiol epoxides but also tetrahydrotetrol epoxides play a dominating role in the metabolic activation of DBA to ultimate mutagens and possibly carcinogens.

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EFFECTS OF 12 AZIRIDINYLBENZOQUINONES ON MITOCHONDRIAL RESPIRATION

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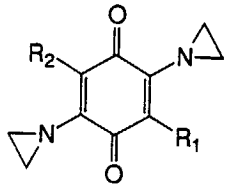

Several 2,5 -bis (1-aziridinyl) - 1,4-benzo-quinones (ABQ) like diaziquone (AZQ), carboquone and triaziquone (Trenimon) have been used in clinical trials as antitumor agents.These compounds can be activated to alkylating species by reduction of the quinone moiety. One-electron reduction by microsomal cytochrome P-450 reductase resulting in the generation of activated oxygen species by redoxcycling could be responsible for acute cytotoxicity .
12 ABQ's (50 or 100 µM) with different substituents in the R1 and R2-position showed a large range of acute toxicity in freshly isolated rat hepatocytes. However, for halogen-substituted ABQ's the time of onset of toxicity in hepatocytes could not be explained by differences in intracellular glutathione depletion. ATP-levels were more important in this respect . Therefore, we assumed that ABQ's could have an additional effect on mitochondrial respiration. In isolated rat liver mitochondria the same 12 ABQ' s were tested for inhibition of the respiratory control ratio (RCR) as measured by oxygen consumption in the presence and absence of ADP. RCR-inhibition correlated well with the electronic field constant F total of the substituents ($n = 12$, $r = 0.94$, $P<0.001$). RCR-inhibition of isolated rat mitochondria was also well correlated with the viability of rat hepatocytes after 120 min. of incubation ($n = 12$, $r = 0.96$, $P<0.001$).
We conclude that, next to GSH-depletion, inhibition of the respiratory control ratio in isolated rat liver mitochondria is a good predictor for acute hepatotoxicity of ABQ's. RCR-inhibition by ABQ's can be predicted adequately from the electronic effects of the substituents.

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PYRIDINE NUCLEOTIDE CHANGES BY AZIRIDINYL-BENZOQUINONES IN ISOLATED RAT HEPATOCYTES

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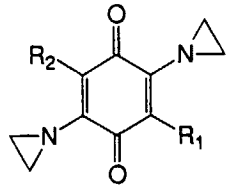

Several 2,5 -bis (1-aziridinyl) - 1,4-benzo-quinones (ABQ) like diaziquone (AZQ), carboquone and triaziquone (Trenimon) have been used in clinical trials as antitumor agents. These compounds can be activated to alkylating species by reduction of the quinone moiety. The acute toxicity of ABQ's in isolated rat hepatocytes is preceded by a fast depletion of glutathione and a decrease in ATP-levels prior to cell death.
Changes in pyridine nucleotide levels could also be important in the cytotoxicity of quinones. Therefore for 3 ABQ's the acute toxicity in hepatocytes was compared at two concentrations and their effects on the levels of NAD^+, NADH, $NADP^+$ and NADPH were studied.
The compounds used were TW 13 (R1 = R2 = H), TW 25 (R1 = Br, R2 = C_2H_5) and carboquone. These compounds caused an initial decrease in NAD^+- and GSH-levels at toxic and non-toxic concentrations. At toxic concentrations the ABQ's caused a more extensive depletion of NAD^+ and a larger and prolonged increase in $NADP^+$ than at non-toxic concentrations. Severe surface blebbing and an initial rise in ($NADP^+$ + NADPH) were observed prior to a decrease in ATP-levels and cell death.
We conclude that in the onset of acute toxicity of these ABQ's a large prolonged rise in $NADP^+$-levels seems to be more important then an initial NAD^+-loss.

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BIOACTIVATION BY GLUTATHIONE CONJUGATION.

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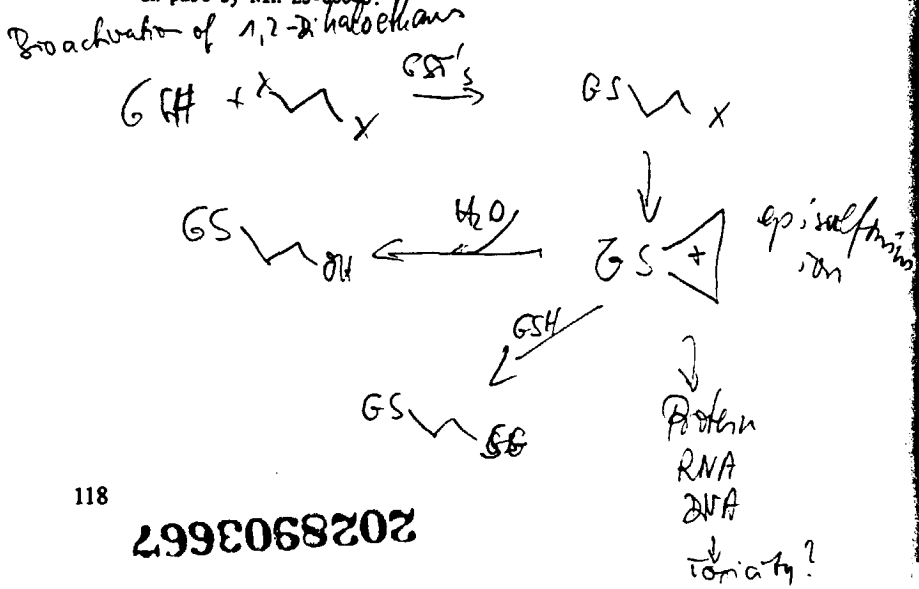
GSH conjugation with drugs and xenobiotics or their reactive metabolites has been traditionally viewed as a metabolic pathway leading to detoxification. The conjugation of GSH with 1,2-dihaloethanes leads to the formation of S-(2-haloethyl)glutathione, which following intramolecular cyclization, produces an electrophilic thiiranium ion. S-(2-chloroethyl)glutathione (CEG) has been identified in the bile of 1-bromo 2-chloroethane (BCE)-treated rats by high-pressure liquid chromatography and precolumn derivatization with o-phthaldehyde. Over a 3-hr interval, 2% of the BCE administered was excreted into the bile as CEG. Comparative metabolism of 1,2-dichloroethane (DCE), 1,2-dibromoethane (DBE) and BCE with freshly isolated rat hepatocytes has allowed the quantitation of GSH consumption, and relative rates of formation of bis-ethylene glutathione, S-(2-hydroxyethyl)glutathione and S-(2-carboxymethyl)glutathione. These studies provide evidence for the extent of formation of CEG during metabolism of these 1,2-dihaloethanes. In vitro alkylation site identification, both the extent and reaction rates of alkylation determined. CEG alkylates a variety of nucleophilic sites commonly found with proteins and nucleic acids and the reaction rate with thiols is more than 10 higher than with any other site tested. The in vivo alkylation of protein thiols groups is currently being investigated for their role in the toxic effects of 1,2-dihaloethanes and the 2-chloroethylnitrosourea tumor drugs. This research has been supported in part by NIH ES-00040.



20289039?

EARLY WARNINGS DURING PHARMACOKINETIC STUDIES WHICH MIGHT INDICATE TOXICITY

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During routine pharmacokinetic and metabolic studies in the drug development program, we sometimes observe warning hints which might indicate toxicity. We divide these warnings in two groups:

1. The first covers the overall fate of the drug, these are kinetic aspects, especially enzyme induction and high affinity to certain biotransformation enzymes.
   Enzyme induction has consequences for pharmacokinetics and toxicity.
   Besides the well known effect of enzyme induction on pharmacokinetics we have compared the results of induction of cytochrome P-450 (parameters: liver weight, total P-450 content, ethoxyresorufinedeethylase, pentoxyresorufine-dealkylase) with the Solt-Farber modell of tumor promotion. Within a class of drug-candidates (eg. imidazopyridines) we find a reasonable correlation between tumor-promotion potency and enzyme induction. Comparing different classes of drugs such a correlation could not be established.

2. The second group of warnings covers the fate of only minute quantities, which may be, however, the toxicological important ones: Indications of reactive intermediates during drug metabolism. These are
   - coloured urine and bile,
   - metabolites generated by the glutathione pathway,
   - binding to macromolecules (e.g. proteins),
   - autoradiographic anomalies.

We present examples how these hints were the first indication of severe liver toxicity of the drug candidate sulmazole.
We further present a strategy how early tests helped us to select other candidates avoiding these serious side-effects.

Source: https://www.industrydocuments.ucsf.edu/docs/sxdl0000

TIENILIC ACID INDUCED HEPATITIS. RESEARCH OF AUTO-ANTIGENS IN RAT LIVER PLASMA MEMBRANES.

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Circulating antibodies directed against liver and kidney microsomes (anti LKM2) were observed in sera of patients with hepatitis induced by tienilic acid (1). Recently, it showed that anti-LKM2 specifically recognize in rat, the cytochrome P-450 UT-A and PB-B (IIC and IIB family respectively) (2).

The in vitro experiments, with rat liver microsomes, show that tienilic acid covalently bound essentially to proteins with a same molecular weight as Cytochrome P-450 UT-A, PB-B and LKM2-antigens. This suggest that the modified protein (haptenized protein) could be the antigen recognized by immune system. Moreover, that involve that the auto-antigen (cytochrome P-450) or an antigenic structure similar to the target antigen to be expressed on the hepatocyte surface.

To clarify this point the untreated, phenobarbital or acid tienilic treated rat plasma membranes were prepared.

The in vitro covalent binding of radiolabelled tienilic acid on plasma membranes seems do not depend on enzymatic activation. Nevertheless, radioactivity is essentially located on 48.5 kD and 51 kD proteins. The distribution of radioactivity between these two proteins depends on rat treatment. The 51 kD radiolabelled protein have the same elecrophoretic mobility than cytochrome P-450 UT-A and also recognize by anti-LKM2 sera.

The preliminary results show the presence of a protein with similar electrophoretic mobility to cytochrome P-450 and LKM2-antigens in these subcellular fractions. Therefore, various hypothesis will be discussed.

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2028903668

STRUCTURE ACTIVITY RELATIONSHIPS FOR CYSTEINE CONJUGATE ß-LYASE FROM RAT KIDNEY CYTOSOLIC FRACTION.

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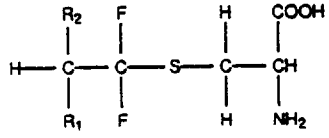
Cysteine conjugate ß-lyase catalyses the C-S cleavage of cysteine conjugates of a number of xenobiotics to yield pyruvate, ammonia and sulphur containing metabolites. The reactive sulphur containing metabolites are held responsible for the nephrotoxicity of some halogenated ethylenes. In addition, the cysteine conjugate ß-lyase enzyme can perform transamination reactions resulting in aminoacid derived keto-acids. As yet little is known about structure activity relationships for cysteine conjugate ß-lyase enzyme. Therefore studies are needed to get insight into the mechanism by which the enzyme catalyses the ß-elimination and transamination reaction. To allow quantitation of both low and high enzymatic activities a new sensitive and selective assay for the simultaneous determination of pyruvate and other α-ketoacids was developed. The method is based on the formation of a flurorescent complex of the α-ketoacids with 1,2-diaminobenzene and subsequent gradient HPLC analysis with fluorescence detection.

The validity of the formation of pyruvate from the cysteine conjugate as a reliable parameter for the ß-elimination *in vitro* was studied by comparing pyruvate formation with thiol formation, using the newly synthesized cysteine conjugate of benzothiazolylchloride. The formed thiol, mercaptobenzothiazole, was analysed by an HPLC assay. Inasmuch as equimolar amounts of pyruvate and mercaptobenzothiazole were formed, it was concluded that the ß-elimination could be satisfactory assessed from pyruvate formation. The extremely low detection limit for pyruvate after derivatization with 1,2-diaminobenzene (12.10^{-9} mol) allowed the determination of enzyme activities as low as 2.10^{-20} mol/min. mg protein.

The ß-elimination activity of the enzyme toward cysteine conjugates of a number of structurally related nephrotoxic halogenated alkenes was investigated with a dialysed rat kidney cytosolic fraction in a TRIS-HCl buffer pH 8.6 at 37 °C. The following compounds were tested :



Code R_1 R_2	
F F	tetrafluoroethylene-cysteine
F Cl	1-chloro-1,2,2-trifluoroethylene-cysteine
Cl Cl	1,1-dichloro-2,2-difluoroethylene-cysteine
Br Br	1,1-dibromo-2,2-difluoroethylene-cysteine

ß-elimination activity decreased in the order, FF>FCl>ClCl>BrBr. Previous studies have shown *in vivo* nephrotoxicity to decrease in the order FF>FCl>ClCl>BrBr.

The structure activity data of these substrates (*in vivo* as well as *in vitro*) were found to be related to the electron withdrawing character of the S- substituent in the order, FF>FCl>ClCl>BrBr.

At present a larger series of cysteine conjugates is tested.

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STUDY ON THE BUTHIONINE SULFOXIMINE-INDUCED GLUTATHIONE DEPLETION IN MICE : ANTAGONISTIC EFFECT OF ANETHOL-DITHIOLTHIONE.

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Previous studies have shown that anetholdithiolthione (ADT) pretreatment exerts a preventive effect on the acetaminophen (AAP)-induced lethality and hepatotoxicity in mice (1). The hypothesis of a mechanism of action mediated by a glutathione-dependent phenomenon had to be considered and supports the present study using buthionine sulfoximine (BSO), a glutathione depletor via gamma-glutamylcysteine synthetase inhibition.

Female Swiss mice (25 ±2 g) were used. BSO (0.5 mM/kg) was administered i.p. 3h prior to ADT (10 and 100 mg/kg p.o.). Mice were sacrificed 5h after ADT administration. Cytosolic reduced (GSH) and oxidized (GSSG) glutathione content and glutathione-related enzyme activities (GSSG-reductase, GSH S-transferases, GSH-peroxidase) were measured in the liver of treated and control groups.
As expected, BSO was able to lower glutathione concentrations in mouse liver to approximately 50% of the control level. Cytosolic glutathione-related enzyme activities were also depressed significantly (GSSG-reductase 70%, GSH S-transferases 67%, GSH-peroxidase 65% of the control values). ADT *per se*, given at a dose of 10 mg or 100 mg/kg per os, was able to increase slightly ($p \# 0.05$) the liver reduced glutathione content. When given 3h after BSO, ADT restored completely the liver glutathione content and the glutathione-related enzyme activities. The mechanism of such events seems to be due to an activation exerted by ADT on gamma-glutamylcysteine synthetase, counteracting the opposite effect of BSO.

1- J.-M. Warnet & al., Pharmacol. Toxicol., 1989, 65, 63-64.

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THE ROLE OF METABOLIC ACTIVATION FOR THE CYTOSTATIC ACTIVITY OF DINALINE AND ACETYLDINALINE

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Dinaline [GOE 1734, 4-Amino-N-(2'-aminophenyl)benzamide, provided by Gödecke Research Institute, Freiburg, FRG], attracted interest because of its cytostatic activity in slow growing tumors with less activity in rapid growing tumors. The differential activity suggested a novel mechanism of action [Berger et al., Canc. Treatm. Reports 69,(1985),1415]. This notion was supported by findings indicating that metabolic activation is not involved; most of the drug is excreted as acetyldinaline in the urine. However, the possibility that reactive metabolites are formed for instance by N-oxidation of this arylamine has not been excluded.
We have proposed recently to use hemoglobin adducts as a monitor for N-oxidation of arylamines in vivo [Albrecht and Neumann, Arch. Toxicol. 57,1,(1985)]. The predominant adduct in most cases is a sulfinic acid amide generated by the reaction of nitroso-arenes with the SH-group of cysteine in erythrocytes. Prerequisite for this pathway is the formation of N-hydroxyl-amine most likely in the liver.
In order to prove a role of metabolic activation , [^{14}C] dinaline and [^{14}C]-acetyldinaline [GOE 5549, 4-acetylamino-N-(2'-aminophenyl)benzamid] (0.1 and 0.5 mmol/kg) were orally administered to female Wistar rats and covalent binding to hemoglobin, plasma proteins and liver proteins after 24 and 48 h was measured. In addition distribution of radioactivity into tissues and excretion into urine and feces were determined and urinary metabolites analysed by HPLC and LC-MS.
The hemoglobin binding index [binding(mmol/molHb):dose (mmol/kg)] was 0.8 and 0.8 for dinaline and 0.7 and 0.6 for acetyldinaline at 24 and 48 h, respectively. Total binding to blood and liver proteins was in the same range and comparable for the two drugs. The pattern of urinary metabolites was also very similar. Besides acetyldinaline as predominant metabolite dinaline, diacetyldinaline, N-acetylparaaminobenzoic acid were identified.
The hemoglobin binding index is very low (comparable to 2,4,5-trimethylaniline 0.7 or 6-chlor-o-toluidine 0.6), suggesting that only a very small fraction of the dose is metabolically activated, which is not likely to account for the toxic or antineoplastic effects. A minor role of metabolic activation is also suggested by the low binding to plasma and liver proteins as well as by the kind of metabolites identified.

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Gut Wall Metabolism of Nisoldipine: Evidence for a Relevant Contribution to First Pass Effect in Dogs.

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The calciumantagonistic dihydropyridine drug nisoldipine is subject to a considerable presystemic metabolic elimination. Its bioavailability after oral administration to dogs has been determined to be only 12% in spite of an almost complete absorption [1]. The relative contributions of the gut wall and the liver to the total first pass effect in dogs are investigated in this study. A stable isotope technique was used to obtain significant results in a limited number of animals despite the marked interindividual variation in the disposition of this high clearance drug.

In 4 beagle dogs the common bile duct and the portal vein were cannulated at least 24 h before administration of the drug. A bolus dose of nisoldipine was administered to conscious dogs intraduodenally and simultaneously carbon-13 labelled nisoldipine was infused directly in the portal vein. The total concentration and the $^{12}C/^{13}C$ ratio of nisoldipine and two of its metabolites (M9, M10) [2] were determined in plasma samples of peripheral blood by a GC and a GC/MS method. After intraportal administration the relative bioavailability of nisoldipine as compared to intraduodenal administration was 2-3 times higher, indicating a relevant prehepatic metabolic degradation. The partial elimination processes contribute with bioavailabilities of about 40% (gut wall and/or intestinal lumen) and 30% (liver) to the resulting bioavailability of 12% in the dog. No administration site specific differences were seen in the formation of metabolites. This was confirmed in a further experiment with intraduodenal administration of carbon-14-labelled nisoldipine. No qualitative and only minor quantitative differences were found in the metabolite profiles in plasma samples obtained from portal venous and peripheral venous blood indicating for nisoldipine similar biotransformation reactions in the gut wall and the liver. It is concluded that from an oral dose given to dogs about 60% is metabolized prehepatically, most probably in the gut wall, and only 30% in the liver.

[1] Ahr et al. Arzneim.Forsch./Drug Res. 38, 1093 (1988)
[2] Scherling et al. Arzneim.Forsch./Drug Res. 38, 1105 (1988)

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PREDICTION OF PHENYTOIN CLEARANCE *IN VIVO* FROM *IN VITRO* PHENYTOIN OXIDASE ACTIVITY.

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Phenytoin is a widely used anticonvulsant with a well defined therapeutic plasma concentration window. In man, as well as in rodents, the low Km for the metabolism of phenytoin results in nonlinear pharmacokinetics within the therapeutic dose range. Hepatic cytochrome P450 oxidises phenytoin to produce phenols and dihydrodials via an arene oxide. We have investigated in rats the kinetics of the initial oxidation of phenytoin *in vivo* and *in vitro* using hepatic microsomes and isolated hepatocytes. The use of the *in vitro* data to predict *in vivo* clearance of phenytoin has been evaluated.

A radiometric procedure (Gerber *et al*, J. Pharmac. Exp. Ther. 178 : 567-579, 1971) has been used to characterise the kinetics of phenytoin oxidation *in vitro* using hepatic microsomes and isolated hepatocytes prepared from untreated male Sprague-Dawley rats using standard methodology. Over a range of 0.1-100μM substrate concentrations the kinetics were consistent with two components of enzyme activity - a high affinity, low capacity site and a low affinity, high capacity site. The activity of the low affinity site (as assessed by Vmax/Km ratio) was approximately one order of magnitude greater than the activity of the high affinity site in both systems. The difference between the Km for the high affinity site (0.6 and 19μM for microsomes and cells respectively) was partially due to non-specific binding in cells (58%±1%).

Five separate groups of rats, unanaesthetised, with previously implanted cannulae in the jugular and femoral veins received both an intravenous bolus and an intravenous infusion of [^{14}C]-phenytoin over a three hour time period. Jugular blood samples were specifically assayed for phenytoin and demonstrated that by varying the bolus dose and infusion rate (1.5-10.7 μmoles/h) steady-state concentrations of 1.6-90μM were achieved. The increase in steady-state concentration with increase in infusion rate was disproportionate and could be analysed in terms of a modified Michaelis-Menten relationship with a Vmax of 93 nmoles/min/250g body weight and a Km of 11μM for the high affinity site. When expressed in terms of unbound concentration, the Km and intrinsic clearance of phenytoin was calculated to be 2μM and 47 ml/min/250g body weight, respectively. As with the *in vitro* systems a second low affinity site was evident with an unbound clearance of 7 ml/min/250g body weight.

In vitro data was scaled up to provide prediction of *in vivo* pharmacokinetic parameters by use of microsomal protein yield, hepatocyte yield, liver weight and degree of non-specific binding within the hepatocytes. Between the three systems, there was good agreement in the relative importance of the two classes of sites and reasonable agreement for the high affinity unbound Km. However the scaled Vmax was overestimated using cell data and underestimated using microsomal data. These discrepancies resulted in poor prediction of *in vivo* clearance from *in vitro* data.

EFFECT OF DIFFERENT PROGESTOGENS ON THE METABOLISM OF ETHINYLOESTRADIOL AND OTHER SUBSTRATES BY HUMAN LIVER MICROSOMES

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The major route of metabolism of the oral contraceptive steroid (OCS) ethinyloestradiol (EE_2) is cytochrome P-450 dependent 2-hydroxylation to form the catechol oestrogen 2-hydroxy EE_2. Recent evidence has shown that the reaction is catalysed by cytochromes from the P450IIC, P450IIE and P450IIIA gene families (Guengerich, 1988; Ball *et al.* 1990). The work of Guengerich (1988) highlights P450IIIA4 ($P450_{NF}$) as a major enzyme involved and also shows EE_2 to be a mechanism-based inhibitor of its own metabolism. Mechanism based or "suicide" inactivation can also occur with other acetylenic steroids including progestogens. Since progestogens are used with EE_2 in combined OCS preparations, it is important to know whether a progestogen can inhibit/inactivate the isozyme(s) responsible for EE_2 2-hydroxylation. Recently it has been suggested, on the basis of a study showing higher EE_2 plasma concentrations in women taking an OC containing EE_2 and gestodene compared to those taking EE_2 and desogestrel, that gestodene may have a more pronounced inhibitory effect on EE_2 oxidative metabolism than desogestrel.

We have compared the effects of 5 progestogens, gestodene (GSD) 3-keto desogestrel (3-KD), norethisterone (NET), levonorgestrel (LNG) and norgestimate (NORG) on EE_2 2-hydroxylation and the oxidative metabolism of other drug substrates (cyclosporin, diazepam, tolbutamide) by human liver microsomes. Details of all the enzyme assays have been previously published (Ball *et al.*, 1990; Tjia *et al.* 1989; Back *et al.* 1988; Kitteringham *et al.* 1989). On co-incubation with EE_2, GSD, 3-KD and LNG produced some concentration dependent inhibition of EE_2 2-hydroxylation (maximum 32% inhibition at 100 μM 3-KD). Pre-incubation of progestogens in a small volume (50 μl) incubation for 30 mins in the presence of an NADPH-generating system enhanced the inhibitory potential of all the steroids (at 100 μM inhibition was for GSD 39%, 3-KD 46%, LNG 46%, NET 51% and NORG 43%). Inhibitory effects were therefore comparable and similar to the macrolide antibiotic troleandomycin. The most marked inhibition seen was of diazepam N-demethylation and hydroxylation by GSD (71% and 57% respectively) and 3-KD (62% and 50% respectively).

The results of this study indicate that all the progestogens in common use have the propensity to inhibit a number of oxidative pathways but there is little evidence for one progestogen being more markedly inhibitory than others.

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